JOURNAL OF AGRICULTURAL RESEARCH

VOL. XI WASHINGTON, D. C., DECEMBER 17, 1917 NO. 12

BACTERIAL-BLIGHT OF BARLEY

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INTRODUCTION

For several years especial attention has been given to diseases of barley (*Hordeum* spp.) by the Department of Plant Pathology of the University of Wisconsin. In 1912 a peculiar leafblight was observed at Madison, Wis., at first causing considerable damage in a plot of two-row Montana barley, later appearing on the adjacent common six-row varieties. The general symptoms indicated that it was a bacterial disease, and laboratory studies strengthened this judgment. The disease has recurred in the departmental plots each year since and has been observed elsewhere as well, but it was not until the summer of 1915 that sufficient critical study could be given to justify publication. These field observations and laboratory studies show that this is a widespread disease, capable of producing economic loss, and is caused by a heretofore undescribed bacterial parasite.

APPEARANCE OF THE DISEASE

ON THE LEAVES.—The first evidence of the disease which has been noticed is on the young, green leaves in the form of small water-soaked areas which later enlarge. In nearly all cases the progress is chiefly longitudinal, the invasion being retarded or limited by the veins. Thus, the more prominent lesions may assume the form of somewhat irregular stripes which may extend in certain cases the full length of the blade and sheath, but are apparently terminated at the node. Only in rare cases has a lesion been found on the culm. The most vigorous development results when the invasion occurs along the midvein, owing apparently to the greater thickness and succulence of these parts. In such cases the striped development becomes most pronounced (Pl. B, 2). Sometimes two or more stripes may develop on a leaf, giving an appearance somewhat resembling the so-called "stripe disease" of barley caused by *Helminthosporium gramineum* (*8*, *9*).[1] In addition to these stripe-like lesions, which are the more common, blotchlike lesions may also occur. At times these may involve so much of adjacent leaf tissue as to cause the entire blade, or considerable portions of it, to shrivel and turn

Reference is made by number (italic) to "Literature cited," p. 643.

light brown. Following infection, the invaded tissues sooner or later lose their normal green, passing through translucent yellowish to brownish tints. Translucency is indeed so constant and distinctive a character of the lesions, even in the later brown stage, as to suggest the specific name chosen for the parasite.

If a leaf showing a strong, freshly developed invasion along the midvein and extending down the sheath (Pl. 47, B) is cut sharply across at the ligule and either portion squeezed between thumb and finger, a milky-gray droplet appears on the cut surface. Microscopic examination of this droplet proves it to be teeming with bacteria. If such or similar lesions are cut across and mounted in water, the bacterial extrusion may be readily demonstrated under the microscope. In a rather advanced stage of the lesion the slime expressed between thumb and finger as referred to above may be of the consistency of thick cream, and, when exposed to the air, quickly hardens to a brittle resinous mass.

These facts are the more interesting since one of the distinctive characters of the disease is the occurrence in nature, under favorable conditions, of a bacterial exudate hardening thus upon exposure. Under humid conditions, especially in the early morning, such exudate may appear on either surface of recently invaded leaf tissues as tiny, clouded droplets. These differ from the ordinary droplets of guttation water in their distribution on the lesions, their smaller size, and in the fact that they are distinctly grayish or milky, whereas the guttation water is crystalline clear. If such exudate droplets remain undisturbed they soon harden into yellowish, resinous granules studding the surface of the lesions (Pl. 47, D), but are easily detachable. Frequently these droplets coalesce with each other or with the guttation water and form conspicuous drops (Pl. 47, A) which later may spread over the leaf surface and dry down as a thin, grayish, almost transparent film (Pl. B, 3; 47, C, *b*). This exudate may occasionally be sufficiently abundant and sticky to entrap small insects (Pl. 47, C, *a*). It is noteworthy also that this dry exudate is quickly redissolved in water (Pl. 48, A); and doubtless this characteristic aids materially in the dissemination of the organism as discussed later.

Since the appearance of the leaves striped by this bacterial blight may sometimes resemble the Helminthosporium stripe disease, distinctions are worth noting as follows: (*a*) the Helminthosporium disease is systemic, striping usually all the leaves of the attacked host plant, whereas the bacterial attack is local; (*b*) the water-soaked and translucent appearance, and (*c*) the exudate are peculiar to the bacterial blight.

ON THE HEADS.—In case the flag leaf is badly attacked before the head escapes, the exudate may so seal up the sheath that the head can not escape (Pl. 49, A). In some cases the heads may emerge, but may be variously bent and distorted, and a portion of the grains blighted.

Ordinarily the effects of the disease are not serious enough to be very conspicuous, causing only water-soaked lesions on the glumes like those on the leaves, but usually with less exudate. Such blighting of the glumes does not destroy the grain as a rule, although the kernels may become discolored (brown) and more or less stunted or shrunken. The most important aspect of such attacks, which will be discussed in a later chapter, relates to the possible overwintering and distribution of the disease with such attacked kernels.

COMPARISON WITH SIMILAR PREVIOUSLY DESCRIBED BACTERIAL DISEASES

From the preceding description it will be noted that this bacterial-blight of barley bears some similarities to certain previously described bacterial diseases. There are also distinct differences.

Ráthay's disease of orchard-grass, caused by *Aplanobacter rathayii* E. F. S., (*10, v. 1, p. 171; v. 3, p. 155–160*) and O'Gara's disease of western wheat-grass, caused by *Aplanobacter agropyri* O'Gara (*6, 7*), both have a characteristic exudate which is yellow in color and is produced in much greater abundance than in the case of the disease on barley. While those diseases are most conspicuous on the inflorescences and upper leaf sheaths the disease on barley occurs chiefly on the leaf blades, with distinct lesions, first on the lower leaves, then progressing upward, and usually less prominently on the heads. Culm and head distortions occasionally occur with barley in a manner somewhat similar to those with Ráthay's and with O'Gara's diseases. Futhermore, while the diseases of orchard-grass and western wheat-grass are caused by nonmotile organisms, the disease of barley, as will be brought out later, is caused by a motile organism.

Manns's bladeblight of oats (*4*) is characteristically different, both as originally described and as observed by the writers, from the bacterial-blight of barley treated in this paper. While both diseases produce local infections in a somewhat similar manner, the bladeblight of oats, as far as noted by the writers, is commonly characterized by a rather wide, conspicuous, light-colored, halo-like margin about the lesions on the leaf blades, well shown by Manns (*4, Pl. XIII, fig. 1 and 3*). Characteristic lesions of this type have been produced on oats by inoculating with pure cultures of what the writers take to be *Pseudomonas avenae* Manns, isolated from oats. Thus, the characteristic lesions on oats are distintly different from those on barley, as previously described, the light-colored, halo-like margin not occurring on the barley. The causal organisms are also different. *P. avenae*, as described by Manns and also as isolated by the writers, is white in culture, monotrichous, and pathogenic on oats. The writers find it nonpathogenic on barley. The causal organism of the disease on barley studied by the writers is yellow in culture, monotrichous, and pathogenic on barley, but not on oats. The writers con-

clude, therefore, that the bladeblight of oats as described by Manns is different from the bacterial-blight of barley treated in this paper.

While Manns (*4, p. 104*) supposes that barley is attacked by his bladeblight of oats, this is regarded by the writers as doubtful. He states that:

> Among the barleys grown at the Station, one variety, known as Primus, showed a susceptibility to the disease even more marked than did the Wideawake variety among the oats. The Oderbrucker variety of barley also showed an occasional blade infected. These observations were verified by cultures.

In this statement it is possible that Manns refers to the same bacterial-blight which is now under consideration, since the present writers have observed this blight on both the Primus and Oderbrucker barleys. And, furthermore, his statement that "observations were verified by cultures" does not seem to imply any carefully conducted cross-inoculation experiments.

DEVELOPMENT OF THE DISEASE

The disease will not ordinarily have blighted enough leaves to attract attention until the plants are two-thirds grown. The earlier stages of its development were, however, followed with care in the experimental fields in 1915 and 1916. In 1915 it was first detected in a field of Beldi barley, a common six-row variety. The seed of this was from a crop badly infected in 1914 and saved for this trial planting. A good, vigorous stand was secured, and the plants were 8 to 10 inches high before the first lesions were observed. These were well scattered over the field, and doubtless a few earlier primary lesions had been overlooked, since no trouble has been met in infecting younger plants in the experimental inoculations. From this time on the disease made steady progress, and two weeks later from 2 to 5 per cent of the plants showed dead, blighted leaves, so that even a casual observer would notice it. In 1916 earlier lesions were noted, but the subsequent development was very similar to that in 1915. This probably represents extreme developments in Wisconsin, but, as will be explained later, the disease is capable of producing greater injury under certain conditions.

OCCURRENCE AND HOST RANGE

GEOGRAPHICAL DISTRIBUTION

The bacterial-blight of barley has been noted and specimens collected by one of the writers (A. G. Johnson) in eight different States as follows: Akron and Greeley, Colo.; Ames, Iowa; St. Paul, Minn.; Amsterdam, Bozeman, and Moccasin, Mont.; Williston, N. Dak.; Corvallis, Oreg. Aberdeen, Brookings, and Highmore, S. Dak.; Janesville and Madison, Wis. If the reference to the disease by Manns (*4, p. 104*) from Wooster, Ohio, is included, the bacterial-blight of barley has been found in nine States. This distribution is indicated in figure 1.

HOSTS

This disease has been noted and collected on a large number of strains of barley including some 52 named varieties, in addition to a large number of unnamed hybrids and unclassified imported stocks. It has been definitely observed on the three main groups of barley: namely, the *Hordeum distichum* group (two-row barleys), the *H. vulgare* group (common six-row barleys), and *H. hexastichum* group (erect six-row barleys). These observations are summarized in Table I.

In connection with these studies the question has been constantly kept in mind as to the occurrence of this or like diseases on other grains. As a result similar bacterial diseases have been found on wheat (*Triticum* spp.), spelt (*Triticum spelta*), rye (*Secale cereale*), timothy (*Phleum pratense*),

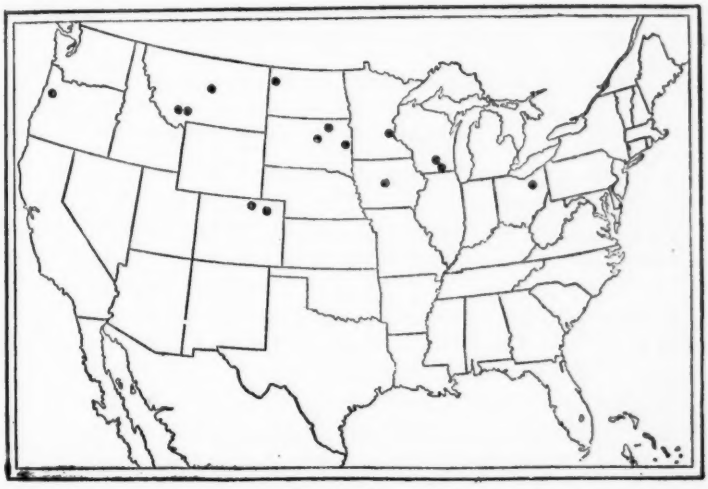

FIG. 1.—Outline map showing known distribution of bacterial-blight of barley in the United States.

and oats (*Avena sativa*). In each case, except that of timothy, the bacterial parasite has been isolated and the disease reproduced by inoculation and the parasite recovered.

With the exception of the bladeblight of oats the symptoms of these diseases are so similar as to be easily considered identical with the barley blight. Detailed study of the organisms, however, shows them to be different from that of barley. Work in connection with these is still in progress, and the results will be presented in subsequent publications.

VARIETAL SUSCEPTIBILITY

Observations have been made in the trial plots of the Office of Cereal Investigations,[1] United States Department of Agriculture, at Highmore,

[1] The writers wish in this connection to express their appreciation for courtesies extended by the various persons concerned at these places.

S. Dak., St. Paul, Minn., and to some extent at Moccasin, Mont., and Williston, N. Dak. (see Table I). In all cases special attention was given to the virulence of the disease on the different varieties of barley upon which it occurred. The differences noted are indicated in the table as follows: "O" is reserved for absence of the disease; "+" indicates very meager occurrence, yet definitely observed; a figure in the numerical range from 1 to 10 is used to represent the degree of infection in any particular plot as compared to that in other plots. As standards "1" is used to represent the condition where infection was very slight—that is, no special search being necessary to find the disease; it being present in small amounts on a considerable number of plants in whatever part of plot noted; "10" is reserved to represent a very abundant infection, where every leaf on every plant was very severely attacked. The intervening numbers between 1 and 10 are used to represent the respective intervening conditions.

TABLE I.—*Bacterial-blight of barley: Varietal range and susceptibility*

Variety.	Highmore, S. Dak.	St. Paul, Minn.	Moccasin, Mont.	Williston, N. Dak.	Madison, Wis.	Akron, Colo.
Hordeum distichum group:						
Benzin		6				
Black Egyptian	5					
Bohemian	5					
Chevalier	4					
Chevalier, Scotch					+	
Frankstein	6					
Golden Melon	5					
Gold Foil	6					
Hanna	6	8		4		
Hannchen	4			4		+
Highland Chief		2				
Holland	6					
Horn	6					
Moravian	7					
Ouchac	4					
Primus	5			5		
Princess	4			4		
Proskowetz	4					
Scholeys	5					
Smyrna		2				
Streigum	5	2				
Svanhals	5					
White Austrian	6					
Hordeum vulgare group:						
Abyssinian		4				
Albacete			6			
Bay Brewing		9				
Beldi	6		8		6	
Benzin		8				
Bernard				4		
Blue		2				
California						+
California Prolific					4	
Caucasian	4					
Chinese Turkestan		9				
Cuzco	6					
Daniel's	4					

TABLE I.—*Bacterial-blight of barley: Varietal range and susceptibility*—Continued

Variety.	Highmore, S. Dak.	St. Paul, Minn.	Moccasin, Mont.	Williston, N. Dak.	Madison, Wis.	Akron, Colo.
Hordeum vulgare group—Con.						
Eagle		8				
Featherston	6	2				
Gatami				5		
Luth	4	2				
Manchurian		2				
Minnesota 105				4		
Oderbrucker	4			2	+	
Odessa	4	6				
Peru	4		8			
Poda	4					
Red River	4					
Summit	+					
Williston C. I. 882				4		
Wisconsin 5					+	
Wisconsin 14	4					
Hordeum hexastichum group:						
Mariout	6		3			
Williston Selection 17				+		

While, at each of the above-mentioned places, a number of other varieties of barley were observed to be free from bacterial blight, a possible indication of some degree of resistance, no mention is made of these in Table I, since no experimental tests have been made on them as yet in this regard.

These observations indicate that the bacterial blight is widespread in its occurrence, and is probably to be found in all barley-growing sections of the United States. It seems to be considerably more prevalent and destructive in the Dakotas than farther east. It attacks all three groups of barley without evident preference. Within a group there is evidently a difference in relative susceptibility. For example, in the *vulgare* group, Oderbrucker seems to be less susceptible than Beldi, and in the *distichum* group, Hanna seems somewhat more susceptible than Chevalier. The observations here recorded were, however, not sufficiently extended to justify any close final comparisons as to such varietal rankings in regard to relative disease resistance.

THE ORGANISM

ISOLATION AND REINFECTION

The freshly invaded tissues as well as the exudate are teeming with bacteria and, after acquaintance with the specific organism, little difficulty has been experienced in isolating it from either source. As illustrating method and results, the following may be cited: A strong, succulent, lower leaf was selected showing natural midvein infection extending down past the ligule. This lesion was in the earlier stages of rapid development and showed the characteristic water-soaked appear-

ance. The leaf was washed carefully in sterile water, dipped into 95 per cent alcohol for a few seconds, and rinsed well in sterile water. The leaf was then cut across with a sterile scalpel at the ligule where the midvein is thickest. This thickened portion, when cut thus, exposed two comparatively large, interior surfaces practically free from external contaminations. By gentle pressure on the midvein a small drop of the milky gray bacterial slime was forced from the interior to the cut surface. This was touched with a sterile platinum needle and agar strokes made. Eight such cultures were made and, within three days at room temperature, these gave similar growths, all apparently dominated by one organism. Plates poured from one of these cultures gave abundant colonies of one type, and several pure cultures were obtained from these. All proved to be the same organism with the characteristic cultural characters and all proved to be pathogenic when sprayed on young barley plants. Infections similar to the natural ones resulted in all cases from such inoculations, and the organism was reisolated. Control plants remained healthy.

The organism has also been obtained, although with more admixture of other species, by each of the following methods:

(1) Similar surface washing and disinfection have been made of invaded leaf tissues. These were then cut into pieces and laid upon the surface of nutrient agar. Abundant bacterial growth spread promptly from the cut edges of such leaf sections. This proved to contain a mixture of species, but from it the parasitic organism has been isolated by making further streak and dilution cultures.

(2) The surface exudate from diseased tissues is teeming with bacteria. Isolations have been made from both fresh and dried exudate at various times. While other organisms may occur, the pathogenic species is readily secured from the exudate in either condition. Isolations have been made from fresh exudate taken directly from diseased plants in the field, also from such plants when the exudate has been forced out in the moist chamber and from plants artificially infected in the greenhouse.

In the same way the organism has been obtained from the hardened granules of the exudate. In one case the granules were picked off the leaves, placed in sterile water blanks, in which they quickly soften and diffuse, and from these successful platings were made. In another case small exudate granules were planted directly on agar plates and the organism recovered from the resulting growth.

(3) The organism has been secured also from the invaded leaf tissues which had been kept as dry herbarium specimens for eight months. Small pieces cut from the lesions were immersed directly in bouillon for one-half hour and platings then made from this. Pure cultures of the organisms were thus obtained and pathogenicity proved by inoculations.

(4) The organism has also been isolated from old barley kernels. On July 18, 1916, pieces of glumes from infected barley 2 years old were

incubated for two hours in bouillon. The organism was subsequently obtained from this by platings and its pathogenicity proved by inoculations (Pl. B, 4; 49, B).

Further details regarding inoculation experiments are given in a later chapter.

MORPHOLOGY

Both in the host plant and in culture media the organism is a short, actively motile rod, usually single or in pairs except in nutrient peptonized beef broth containing 2 per cent of sodium chlorid, in which it forms long nonmotile chains. Growing on peptone beef agar (3-day-old cultures), the organism measures 0.5 to 0.8 by 1 to 2.5 μ when stained with gentian-violet or Ziehl's carbol-fuchsin. It stains less readily with Loeffler's methylene-blue.

No spores have been definitely seen. Capsules are formed on agar and in milk. The organism is motile by means of a single polar flagellum. Repeated stainings for flagella both by V. A. Moore's modification of Loeffler's method and by Zettnow's method agree in showing the monotrichous character of the organism (fig. 2). It is Gram-negative and not acid-fast.

FIG. 2.—*Bacterium translucens:* from 96-hour growth on potato agar, stained by Zettnow's method to show flagella. × 2,000.

CULTURAL CHARACTERS

Unless otherwise specified, these have been studied at approximately 25° C., a temperature favorable for the organism. Reference to reaction of media is made in terms of Fuller's scale. Unless otherwise noted all reactions were determined by titration with phenolphthalein as indicator, following boiling of solution tested. Notations of color have been made in comparison with Ridgway's Color Standards.[1]

AGAR POURED PLATES.—On peptone-beef agar, reaction +10, colonies appear in 48 to 60 hours and at end of four days are 1 to 5 mm. in diameter, circular, smooth, shining, and amorphous except for inconspicuous somewhat irregular concentric striations within the colonies.[2] Margin entire. Colonies are wax-yellow tinged with old-gold in color, soft but not viscid in consistency. Buried colonies are lenticular or granular (Pl. 48, C).

AGAR STABS.—Stabs in +10 peptone-beef agar when 3 days old show a raised, smooth, shining, wax-yellow, opaque surface growth 5 mm. in diameter. Growth is only at the surface.

[1] RIDWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Bibliography, p. 42–43. 1912.

[2] These striations within the colonies are evident only under slight magnification, about ×10, and under favorable lighting; semidirect lighting best. These striations are so inconspicuous as to have been overlooked by the writers until Dr. Erwin F. Smith, of the Bureau of Plant Industry, called attention to similar colony structure in a closely related organism.

AGAR SLANTS.—On peptone-beef agar slants, stroke cultures make a moderate, filiform, wax-yellow, opaque, smooth, shining, convex growth.

GELATIN PLATES.—Surface colonies show a characteristic margin. This may be due to liquefaction, which takes place slowly at 18° C. In about 10 days liquefaction is complete and the colonies float.

GELATIN STABS.—At 20° C. in +10 peptone gelatin the surface growth is abundant after several days with a medium pit of liquefaction. At the end of four days the growth becomes stratiform and slowly liquefies its way toward the bottom of the tube. It progresses somewhat more than halfway in 40 days.

BEEF BOUILLON.—In +10 peptone-beef bouillon uniform clouding occurs within 48 hours. This clouding subsequently becomes rather strong; a membranous pellicle forms at about the fourth day and then slow clearing begins, becoming first evident just under the pellicle. Sediment is flocculent. Crystals have been noted.

POTATO CYLINDERS.—Growth on steamed potato cylinders in two days is abundant, spreading, slimy, flat, glistening, smooth, without odor, and in color wax-yellow with a tinge of old-gold. Later the growth becomes very abundant and the elevation convex, but no change in medium is noticed at the end of two weeks.

MILK.—Inoculated milk coagulates slowly and then clears, beginning at the top. In a month there is partial digestion, some soft coagulum, and a heavy, watery, yellow growth at the top. Reaction showed increase in acidity.

LITMUS MILK.—No marked color change is noticeable during the first two weeks, although a heavy yellow growth develops at the top. Then reduction begins, and in a month the medium is amber-colored.

FERMENTATION TUBES.—The tests were made in 2 per cent Witte's peptone water and 2 per cent, respectively, of each of the following carbon compounds: Dextrose, lactose, saccharose. maltose, glycerin, and mannit. Clouding was first noted at end of two days. At end of six days nearly all were clouded in the open end. Mannit and glycerin were the slowest to cloud. In all cases there was a definite line of demarcation across the inner part of the U and no visible growth in the closed arm. No gas was formed in the closed arm in any case.

When 20 days old, they were titrated with phenolphthalein as indicator, boiling to drive off carbon dioxid. In each case there was slight acid production. They were similarly tested again when 30 days old, and found to have produced more acid, When the test was made without boiling, they were found to be almost neutral to phenolphthalein. Both ammonia and acids are produced, the ammonia apparently slightly in excess.

A strong test for ammonia was found by Nessler's reagent and also by testing the gas evolved with red litmus paper when a portion of the culture was heated with potassium hydroxid.

No gas was formed in fermentation tubes containing sterile milk. The milk in the open end cleared gradually, followed later by the slow clearing in the closed end. The reaction was acid to litmus.

NITRATE BOUILLON in fermentation tubes gave a good clouding in open end; none in closed end. No gas was produced. There was a strong test for ammonia and nitrates. Since the organism produces ammonia from peptone, the test does not show whether nitrates were reduced or not. There was a slight production of acid when tested after boiling. No nitrites found.

TOLERATION OF SODIUM CHLORID.—Neutral peptone-beef bouillons containing, respectively, 2, 5, 6, and 7 per cent of chemically pure sodium chlorid were inoculated from young bouillon cultures. There was uniform clouding after four days in the 2 per cent strength. All other strengths inhibited growth. The experiment was repeated, using 2, 3, and 4 per cent of sodium chlorid. Growth occurred in the 2 per cent strength only, and in this the organism developed long chains and soon died.

OPTIMUM REACTION AND TOLERATION LIMITS.—Peptonized beef bouillon was used with sodium hydrate as the alkali under test and hydrochloric as the acid. Bouillons were prepared titrating +25, +20, +10, +5, 0, −20, and −30, and uniformly inoculated from a 4-day-old bouillon culture. At the end of 48 hours, growth was evident in +10, +5, and 0. Clouding was weak in the 0 bouillon at this time, but the +5 and +10 were heavily clouded. At the end of four days a pellicle was evident on the +10 tubes. The +5 later formed a weak pellicle, but none formed on the 0. Gradual clearing began on the fifth day, but the cultures never cleared entirely. The sediment in the +10 was somewhat flocculent, but in the +5 and 0 it had more of a slimy, stringy consistency. None of the other reactions showed any growth.

It seems evident, therefore, that +10 Fuller's scale represents the optimum reaction and that the organism is highly sensitive to both alkali and acid.

Another test was made of acid toleration, using +10 bouillon as the basic medium, to which was added 0.1 and 0.2 per cent, respectively, of malic, tartaric, and citric acids. The cultures were kept under observation for 20 days following inoculation, but no growth was observed in any of the acidulated tubes.

USCHINSKY'S SOLUTION.—A moderate, uniform clouding was noticeable on the fifth to eighth day. The clouding never became heavy and no pellicle was formed. There was slight change in the medium. Only a slight yellow tinge was given to it and a small amount of yellow sediment. The bacteria formed long chains in this medium.

FERMI'S SOLUTION.—No growth.

COHN'S SOLUTION.—No growth.

STARCH AGAR.—No evidence of diastasic action on potato starch suspended in peptone-beef agar, tests being made with potassium iodid-iodin.

REDUCTION OF NITRATES.—Cultures in nitrate bouillon were tested with Trommsdorf's reagent, first after 24 hours and repeatedly thereafter, and gave negative tests for nitrites. At the end of 30 days the cultures gave strong tests for ammonia and nitrates but negative test for nitrites. It is doubtful if nitrates are reduced, since good tests for ammonia are obtained from bouillon which does not contain potassium nitrate.

INDOL.—Tests in peptonized Uschinsky's solution and in peptone water at the end of two weeks and four weeks showed slight indol production. *B. coli* run in comparison showed much stronger indol production.

METHYLENE-BLUE IN MILK.—Reduction is slow, first evident at end of three weeks. At end of two months the cultures are white except for a surface layer and some sediment which is blue.

BLOOD SERUM.—Stroke cultures on Loeffler's blood serum gave a moderate, wax-yellow, glistening, smooth, convex, filiform growth. Medium not liquefied.

AEROBISM.—The organism appears to be strictly aerobic. It does not grow in the closed end of fermentation tubes with any of the carbon foods tested. No growth occurs in the lower end of stab cultures of agar or gelatin. Shake cultures of agar showed growth at or near the surface only.

LITMUS AGAR WITH SUGARS.—On litmus-lactose agar stroke cultures there is abundant growth and the medium becomes somewhat bluer, probably due to production of acids and ammonia, the ammonia a little in excess. The same may be said about the growth on litmus-maltose agar.

On litmus-dextrose agar stroke cultures there is very abundant growth, slight bluing along the stroke, reduction to an amber color in two weeks.

Optimum reaction for growth is +10 Fuller's scale.

TEMPERATURE RELATIONS

In +10 peptone beef bouillon the optimum temperature was 26° C. or slightly above. No growth was secured at 36°, and only part of the tubes clouded at 35°. Slow growth occurred at 10°; none at 6°. Nu-

merous trials show the thermal death point of 2-hour cultures made from 48-hour cultures to be apparently about 50°. In the case of old cultures growth has been obtained and pathogenicity of organism proved after a 10-minute exposure to 55°, indicating the development of a slightly more resistant type of cell under these conditions.

DESICCATION

The organism is highly resistant to drying as it occurs normally in the host tissues, since, as previously explained, it has been isolated from dried herbarium specimens of leaves 8 months old and it may live on the seed at least two years. It also survives long on culture media. When dried on sterile cover glasses it seems more sensitive. Thus, a young well-clouded bouillon culture was diluted with equal parts of sterile water and a full 2-mm. loop transferred to each of a series of sterile cover glasses and these dried in a sterile chamber. When tested by plunging these in broth at intervals, it was found that most perished within 24 hours, and all within two days.

SUSCEPTIBILITY TO MERCURIC CHLORID

Various tests have shown the organism to be very sensitive to mercuric chlorid.

TECHNICAL DESCRIPTION

On the basis of the foregoing studies the parasite in briefly characterized as follows:

Bacterium translucens, n. sp.[1,2]

Cylindrical rods rounded at ends, solitary or in pairs; individual rods 0.5 to 0.8 by 1 to 2.5 μ, motile by a single polar flagellum; aerobic, no spores.

Superficial colonies in peptone-beef agar plates round, smooth, shining, amorphous except for inconspicuous somewhat irregular concentric striations within, wax-yellow tinged with old-gold; margin entire.

Liquefies gelatin slowly; produces slight acidity in milk; digests casein; nitrates not reduced; acid produced in cultures with various sugars. No gas produced. Gram-negative. Group number 211.2222532.

Pathogenic in leaves of *Hordeum vulgare*, *H. distichum*, *H. hexastichum*, forming translucent elliptical to striaform lesions.

Type locality: Madison, Wis., on *Hordeum vulgare*.

Distribution: Northern Mississippi Valley and westward to Pacific coast.

[1] According to Migula's classification, the combination would be *Pseudomonas translucens*, n. sp.

[2] **Bacterium translucens,** sp. nov., aerobium, asporum; coloniis cum striis inconspicuis internis aliquid irregularibus concentricisque; baculis cylindricis apicibus rotundatis, solitariis vel binis; baculis singulis 0.5—0.8×1.0—2.5 μ, flagello uno polare mobilibus.

Coloniae superficiales in agar-agar rotundae, leves, nitentes, melleae. Liquefacit gelatinam lente. Lac sterile acidum facit; casein segregat. Nitrum non redigit. Culturae in mediis cum saccharo sacchari, saccharo uvae, saccharo lactis acidae fiunt. Gas non facitur. Methodo Grami non coloratur.

Habitat in foliis vivis *Hordei vulgaris*, *H. distichi*, *H. hexastichi* in lineis vel maculis translucentibus. Madison, Wisconsin. Amer. bor.

INOCULATION EXPERIMENTS

ON BARLEY.—The disease has been reproduced on barley with the characteristic symptoms by artificial inoculation and the original organism recovered. While a considerable percentage of infections have resulted where wound inoculations were made, the best results and those leading to the typical lesions have followed the spraying of the organisms in watery suspension upon young uninjured leaves.

The following details of one of the earliest series of inoculations illustrates this:

Beldi barley was sown in 6-inch pots, and on June 28, 1915, when the vigorous young growth was about 4 inches high, four uniform pots, each containing about 25 plants, were selected for experimental uses. Of these pot I was inoculated by atomizer spray with a water suspension of the organism obtained as follows: A pure culture of the organism was plated out from the milky ooze squeezed from a typical leaf midvein lesion. Sterile water was poured into an agar tube streak culture and the bacterial growth allowed to diffuse into this, which was then used to spray upon the young barley plants. In pot II a wound inoculation was made on the first green leaf of each plant. This was done by touching with the needle the bacterial growth of a young agar streak culture of the same source as described above. With this needle a slight wound was made about midway the leaf, in the midvein or near it, the attempt being to puncture but one epidermal layer and to avoid a perforation through the entire leaf. In pot III wound inoculations were made as in II except that the inoculation needle was dipped directly into the milky ooze squeezed from a typical barley leaf midvein lesion. Pot IV was held as a control, the plants being wounded as in II and III, but with sterile needle.

These pots of barley plants had all been grown in the greenhouse previous to inoculation and were kept there in a damp chamber for 24 hours following this, then transferred to the open garden. The pots were here sunken to the soil level and thus given normal outdoor exposure, the weather being warm and rather dry.

Infection was noted in pots I, II, and III on July 7, an incubation period of nine days. There was no infection in IV. On July 9 pot I showed approximately 100 per cent of plants infected, II approximately 40 per cent, III about 20 per cent, and IV no plants infected. After the occurrence of splashing rains, secondary infections appeared, and at the end of a month, when these were at their height, pots I, II, and III showed a like condition, with abundant lesions on practically every plant, whereas the control, pot IV, showed only four or five recently developed lesions. Since pot IV was only 18 inches removed from the nearest infected pot and fully exposed for the month, splashing rains were held responsible for these few late infections.

On July 7 wound inoculations were repeated (Beldi barley, 4 inches high), making a single prick with a very fine needle into a single marked leaf on each plant in the pot; plants were transferred to the open garden immediately following inoculation, no artificial means being used to increase aerial moisture. About 90 per cent of infection resulted.

In another series of inoculations, in which pot-grown greenhouse plants were used, a small drop from a young broth culture of the organism was placed on each leaf and the epidermis underneath punctured in one place with a fine-pointed needle. The plants were kept in the greenhouse in a rather humid atmosphere. Water-soaked infection areas appeared just above the pricked spot in four days, one of the shortest incubation periods we have noted. This series gave 100 per cent of infection.

Barley was planted in rows in the open garden on July 15. On July 22, when the seedlings were about 1 inch high, they were inoculated by spraying with a water suspension of pure culture. No shading or cover was used, but the weather was moist. On July 28 the first infections were noted, an incubation period of six days under normal outdoor conditions. On July 30 the lesions were numerous and typical, with an abundance of liquid exudate (Pl. 47, A).

In other cases abundant infections have been obtained by similarly spraying older plants (8 to 25 days) in the open without subsequent covering.

These results show that infection is easily secured under normal environmental conditions without artificially rupturing the epidermis. It is noteworthy that the application of the organisms with the atomizer insures better results than wound inoculation. Probably this is due to the rapid drying out of the wounded tissues when the puncture method is employed. Another interesting outcome is the lesser percentage of success where the inoculations were made by direct transfer of the bacterial ooze from infected tissues as compared with pure-culture inoculations. This is possibly due in part to the low degree of vitality of the organisms in such direct transfer. It may be in part attributed to the fact that such ooze hardens very quickly upon exposure, thus imprisoning the organisms temporarily at least; and meanwhile the tissues in the wounded spot dry out to a degree unfavorable to infection.

ON OTHER GRAINS.—Several trials were made to infect other cereals with this organism. Two series of inoculations were run on barley, wheat, rye, oats, spelt, emmer (*Triticum dicoccum*) einkorn (*Triticum monococcum*), and timothy, and a number of different series on barley, wheat, rye, and oats. All gave uniformly negative results except in the case of barley which was in every instance heavily infected. Cross-inoculations with this and other cereal-blight organisms have been briefly reported previously (*1*), and work in connection with these is still in progress.

RELATIONS TO HOST TISSUE

A study of razor sections of the lesions as they occur in fresh leaves show the general facts to be as follows: The bacterial invasion is in the parenchyma. The parenchyma cell walls become disintegrated and in the interior tissues long rifts result, which extend lengthwise of the leaf blades. In such lesions the bacterial slime fills the interior cavities, expelling the air; hence, the translucency of the invaded areas. Later this bacterial slime hardens to a resinous texture, making the translucent condition a permanent characteristic of the older lesions.

For detailed histological studies material was secured from recently infected young barley leaves. These had been inoculated by spraying with a water suspension of a pure culture and showed numerous small translucent infection areas. Pieces of these were fixed in boiling absolute alcohol saturated with mercuric cholorid, embedded in paraffin, and sectioned. Such sections stained with carbol-fuchsin showed infections to be stomatal. In some cases the invasion was as yet confined to the stomatal chamber which might be only partially occupied or crowded full of the organisms. Numerous cases showed intercellular invasions in the parenchyma in the early development of the lesions. In other cases later stages were in evidence where the bacteria had developed in large masses, evidently spreading from the stomatal chambers through the intercellular spaces, crowding apart and crushing the parnchyma cells. Instances of advanced invasion were found where the cell walls had partially disappeared, but the details of wall solution have not received a critical study as yet. In the older lesions, as noted above, there occurred long cavities filled with the bacterial growths. It is from such cavities in the advanced stages of the lesion that the bacterial slime may be squeezed out which has been the source of material for the isolation and inoculation work previously described.

OVERWINTERING AND DISSEMINATION

The fact that the disease may attack the heads early in their development has already been pointed out. The glumes may show water-soaked areas like those on the leaves and, upon these, drops of the bacterial exudate may appear and harden into tiny resinous granules or spread as a glistening film. Observation of these facts at once suggested two questions, each of much importance. (1) How much is the grain checked or injured by such attack? (2) Does the organism overwinter on such grains or other barley tissues?

As to the first, the writers' observations have failed to detect any case where the grains are killed, although in certain cases there may be considerable injury. Although they may be more or less shrunken, they generally mature and are viable. This seemed somewhat surprising, in view of the extent of the damage to the leaves, but is apparently explained in part by the fact that the heads are susceptible for so brief a

period. The only cases observed where the heads were destroyed have occurred when the flag leaf and its sheath were badly attacked while the head was still inclosed. The bacterial exudate may then so seal the developing sheath that the normal escape of the head is impossible and these culms can not "head out" (Pl. 49, A).

As to the overwintering of the organism, evidence has been obtained in two ways: First, blighted leaves showing the well-developed translucent lesions, which had lain in the laboratory as dry herbarium specimens for eight months (July-March), were used as material from which to make poured-plate isolations. From these the characteristic organism was secured, its identity determined by cultural characters and its pathogenicity by inoculations on young barley leaves. Apparently the organism lives longer in the interior of these desiccated leaves than it does in the dried exudate upon the surface, since poured plates from the exudate granules from similarly stored leaves failed to show the organism. Only the smaller granules were available for such trial, and further tests may show that in the larger exudate granules the organism may persist longer.

The second and more direct evidence relates to the persistence of the organism with the seed grains which may be stored over the winter or disseminated widely. Field evidence indicated early that in certain cases the disease was introduced with seed from certain western sources. In following up this matter seed was collected in the summer of 1914 from a field of Beldi barley in Montana severely attacked by the blight. Lesions were evident on the glumes of these plants before maturity and showed, although less clearly, upon the ripe grain.

Some of this seed was planted in the trial grounds at Madison in the spring of 1915. The bacterial-blight was detected on the leaves in this plot when the plants were 8 inches high, scattered infections then occurring throughout the plot. At the same time no disease could be detected on the other barley plots in series which were planted with Wisconsin-grown seed supposed to be free from the disease. Later in the summer, however, the blight was produced by inoculation upon certain of these others, indicating that the difference was not primarily in varietal susceptibility.

As previously reported (*3*), isolation cultures were made in July, 1916, from the glumes of some of this same infected grain, which was collected in Montana in 1914. The characteristic barley-blight organism was found to be present, was secured in pure culture, and its continued pathogenicity was proved by inoculation experiments (Pl. 49, B). It is thus apparent that the organism may persist with the stored grain and remain pathogenic after at least two years of dormancy.

The conclusion that the disease is introduced with the seed seems further justified by the writers' observations in preceding years. Thus, in 1912, it first appeared in a plot of Chevalier, a two-row barley, the seed of which was brought from Montana. Later it developed on the

adjacent six-row varieties, having evidently spread from the Chevalier plot. The following season this same plot of soil was replanted with clean barley, and none of the disease reappeared. Our conclusion is, therefore, that probably under Wisconsin conditions the disease usually comes from organisms overwintering with the seed rather than in the soil, although the organism is capable of living over the winter in the dry, blighted straw.

It is doubtless carried with the seed to new localities, and not only its overwintering but its long-range dissemination is thus accounted for. For local dissemination in the field water is apparently the chief agent, although thrips and aphids may possibly play a considerable part. As already shown, surface application of the organism by spray or otherwise without wounding suffices for infection. The abundant bacterial exudate from blighting tissues is readily distributed by beating rain or trickling dew and may doubtless be carried farther, though in lesser amounts, by visiting insects. The prompt hardening of this exudate into resinous masses preserves the vitality of the organism for a long time without hindering this mode of water dissemination, since the dry exudate again quickly softens and diffuses upon the application of water. (Pl. 48, A.)

CONTROL MEASURES

It is not practicable to exercise any control measures aiming to check the spread of the disease after it appears in the barley field. On the other hand, the conclusion of the writers that its overwintering and long-range dissemination occur chiefly, if not wholly, with the seed encourages the hope that practical control measures may be developed.

Ordinarily it may suffice to avoid the use of seed from badly infected fields or localities when these conditions are known. In case of doubt it seems probable that seed disinfection may greatly reduce the danger of introducing the disease, if not eliminate it altogether. The writers have not as yet had opportunity for a convincing trial of this matter. An experiment bearing upon it was, however, made in March, 1916, as follows: Barley grain harvested from an infected field in 1914 had meanwhile lain dry in the laboratory. Isolations from the glumes then (1916) showed the organism still alive. After soaking this grain for two hours in formaldehyde solution (1 part of Merck's 40 per cent formalin in 320 parts of water) attempts to isolate the organism from the material failed. These results should not, however, be given too great weight, since negative results had also been obtained with some samples of untreated seed suspected of harboring the organism. Owing to the high degree of sensitiveness of the organism to mercuric chlorid, it is probable that this will be even more reliable for use as a seed disinfectant. The hot-water method may also prove useful, especially if later studies show that the organisms penetrate the kernel.

SUMMARY

(1) Bacterial blight of barley is a widespread disease capable of producing serious loss and is caused by a heretofore undescribed parasite.

(2) The lesions on the leaves begin as small water-soaked areas which enlarge to yellowish or brownish somewhat translucent blotches or irregular stripes. Similar lesions may appear later on the glumes. The chief injury, however, is apparently to the foliage.

(3) A bacterial exudate may appear scattered over the lesions as tiny clouded droplets which harden into yellowish resinous granules or spread to form a grayish, flaky surface film. This exudate and the translucency of the invaded parts are characters distinguishing the bacterial blight from the Helminthosporium diseases.

(4) This disease bears some resemblance to Ráthay's disease of orchard grass, to O'Gara's disease of wheat grass, and to Mann's blade blight of oats. It is, however, distinct from any of these.

(5) Primary lesions may appear on seedling plants very early in their development and secondary lesions when the plants are 8 to 10 inches high. Later the disease spreads with increasing rapidity.

(6) The disease has been found widely distributed from the eastern Mississippi Valley to the Pacific coast. It attacks the barleys of all three of the main groups: viz, two-row, common six-row, and erect six-row. Within each group there is a considerable range of varietal susceptibility and, so far as observed, some are free from attack, but further work is necessary for final comparisons.

(7) The invaded tissues and exudate are teeming with the bacterial parasite which has been isolated not only from these sources but also from dry overwintered leaves and from grain 2 years old. The organism is a monotrichous rod, yellow in culture, with the group number 211.2222532 and described as *Bacterium translucens*, n. sp.

(8) Inoculation experiments have shown that the disease may be readily induced on barley by spraying with water suspensions of the organism. Negative results were obtained from inoculations on oats, rye, wheat, spelt, emmer, einkorn, and timothy.

(9) The channels of invasion are stomatal and intercellular.

(10) Although the organism may overwinter in infected leaves, diseased kernels doubtless constitute the chief means of dissemination and source of spring infection.

(11) While control measures are not as yet fully worked out, the most promising methods consist in the avoidance of infected seed and in seed disinfection.

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PLATE B

1.—Healthy barley leaf for comparison.

2.—Early stage of bacterial blight (natural infection) showing a translucent, water-soaked stripe along the midrib, yellowing toward the apex. Often such lesions as this will be studded with exudate granules (see Pl. 47, D) or show a drop of bacterial ooze at the base (see Pl. 47, B).

3.—More advanced stage of bacterial blight of barley (natural infection) where numerous local invasions have developed. Note the many water-soaked spots; the glistening grayish exudate scales below, which are shown in sharper color contrast in this painting than in nature. The tissues, as in the more advanced lesions above, commonly retain their translucence as they yellow and die because of the hardened bacterial ooze retained within.

4.—Barley seedling showing abundant infection spots on the lower leaf from atomizer inoculation with strain isolated from old infected seed. The individual greenish water-soaked spots represent stomatal infections. The general dying and yellowing above resulted from such invasions.

5.—Barley seedling similarly infected with strain isolated from blighting leaf.

1
2
3
4
5
Shull

mm
A
B
a
b
mm
C
mm
D

PLATE 47

A.—Barley leafblight, × 5½, natural infection, showing exudate gathered in three grayish glistening drops on the surface of the water-soaked blighting area.

B.—Blighting barley leaf, × 1½. Early stage of mid leaf stripe invasion, this stripe showing the water-soaked translucent appearance; the grayish exudate so abundant as to have formed a glistening droplet just above the ligule.

C.—Blighting barley leaves natural infection, × 2. *a*, A small insect (midge) entrapped in the exudate. *b*, The dry thin grayish exudate film (X) at the lower part of the blighted area is partly detached and turned back. The exudate film is so nearly transparent that the leaf veins are slightly visible through it.

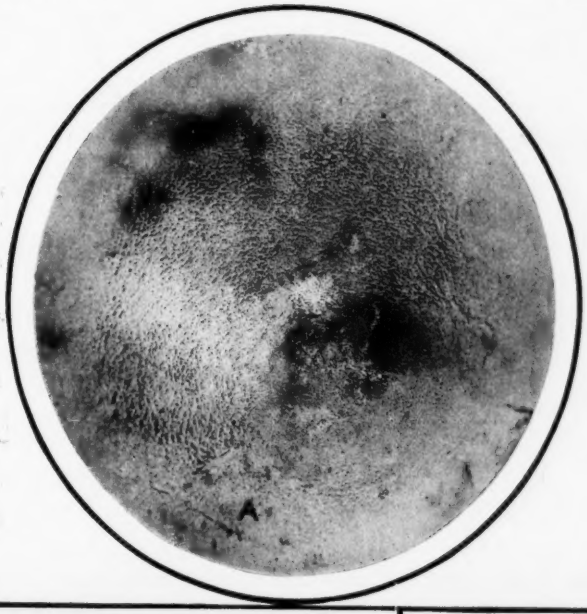
D.—Barley leaf, × 4, showing general blighting, the surface studded with numerous exudate droplets hardened into yellowish resinous granules, readily dislodged. These granules tend to appear in longitudinal rows upon the stripes.

PLATE 48

A.—Photomicrograph of a bead of hardened exudate dissolving in water under microscope. The granular effect is due to the bacteria which quickly diffuse at the margins.

B.—Five-day-old agar slant cultures of *Bact. translucens* incubated at 24° C. *a*, On peptone beef agar. *b*, On potato agar. Upon the latter medium the organism grows profusely and in a characteristic manner—viz, growth heaps up abundantly, is slightly more watery, and a little lighter in color than on peptone beef agar.

C.—Colonies of *Bact. translucens* on peptone-beef agar plate, dilution culture from bouillon, photographed by semidirect lighting to show characteristic internal somewhat irregular concentric striations. × 5½.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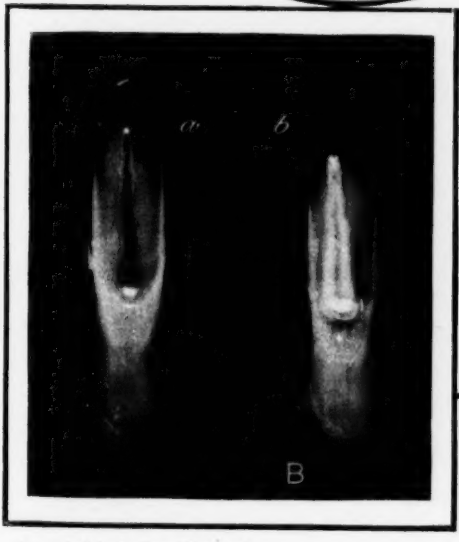
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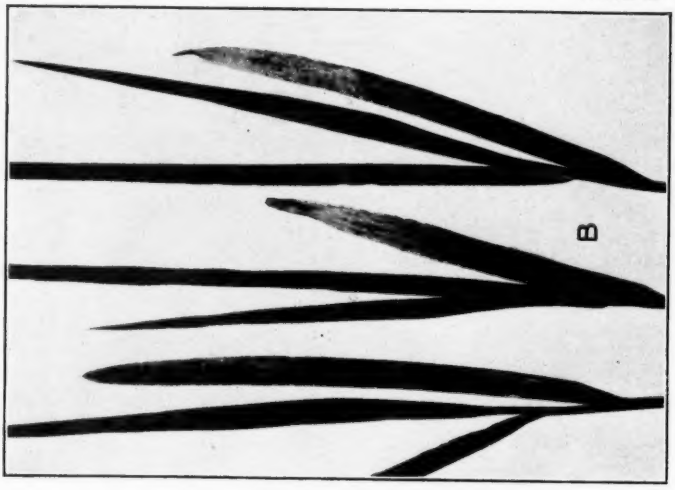
B

C


B

A

PLATE 49

A.—Blighting barley culms at heading stage. The sheaths so sealed by the bacterial exudate as to prevent the escape of the inclosed heads which thus perish undeveloped. × ⅔.

B.—Barley seedlings showing abundant infections on first leaves three days after atomizer inoculation with strain of organism isolated from 2-year-old seed. The photograph was made, using chiefly transmitted light to show the translucent character of the invaded areas. × ⅗.

INFLUENCE OF THE AGE OF THE COW ON THE COMPOSITION AND PROPERTIES OF MILK AND MILK FAT

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INTRODUCTION

The question of the changes in the composition of milk with successive lactation periods has been one which has from time to time attracted the attention of chemists and physiologists. That phase of the question relative to the percentage of fat in the milk has been of great practical importance to the dairyman, who is interested in knowing whether the percentage of fat in the milk of the heifer can be used as an index of what may be expected of the mature animal. Data bearing on this question have been compiled by several investigators, but the conclusions drawn from these data have not been entirely uniform. These data have been gathered in most cases from dairy herds located at various experiment stations, both abroad and in this country.

It is the purpose of the present paper to offer data on this question taken from the records of the University of Missouri dairy herd. It is believed that the data have an added value because of the fact that this herd has always been composed of pure-bred animals only, comprising the Jersey, Holstein, Ayrshire, and Shorthorn breeds, and also because of the fact that all of the animals were born and raised at the Station, with the exception, of course, of the individuals of each breed which were purchased when the herd was started.

Another phase of the question of the influence of age on the composition of milk is that of the effect of old age. Whether or not very old cows, which are not infrequently found in long-established herds or in private families, continue to give normal milk has never, to our knowledge, been studied. A very thorough search of the literature has failed to disclose any investigations of this character. The authors have had an opportunity to study the composition of the milk of one aged cow in the University herd and also of two other cows in the same herd which were advanced in years. The data secured from these animals will also form a part of the present paper.

HISTORICAL REVIEW

The principal investigations of the influence of age upon the percentage of fat in the milk have been by La Cour and by Haas and Högström in Europe and by Wing and by Hills and Hooper in this country.

La Cour (*7*)[1] reported the results of five years' observations of the percentage of cream in the milk of a large number of cows, using the Fjord

[1] Reference is made by number (italic) to "Literature cited," p. 657-658.

control centrifuge. When the results were compiled with regard to the age of the cows, it appeared that the percentage of fat in the milk was highest during the first three years of the cow's lactation life and declined at the rate of about 0.1 per cent of cream at intervals of two to three years. A few very old animals—that is, 15 to 18 years old—showed cream percentage 0.6 to 0.9 per cent of cream lower than normal, which was that observed during the fifth and sixth years of the cow's life.

Haas(*2*) studied the data gathered at Allgaü from 1894 to 1898 from the standpoint of the influence of the age of the cow upon the percentage of fat in the milk. The conclusion drawn from the data was that the fat content increases up to the fifth calf, after which there is generally a gradual decline. Individual animals, however, showed exceptions to this rule. It is stated also that the specific gravity of the milk is highest with the first calf and lowest with the sixth.

Högström (*4*) compared the percentage of fat in the milk of 393 Ayrshire cows of different ages with the average percentage of fat of 799 lactation periods of these cows. The conclusion drawn was that the fat content of the milk is highest at 3 years of age, is high at 4 years, and remains close to the average for the family during the full activity of the cow—that is, through the tenth year—after which the fat percentage again rises slightly. The difference between the highest fat percentage (at 3 years of age) and the average was 0.14 per cent of fat.

Wing (*8*) reported the average fat percentage of individuals in a herd of 25 grade Jersey and Holstein cows from the second to the sixth year with the following results: 3.71, 3.71, 3.68, 3.60, and 3.49 per cent of fat. The data were based on the first 40 weeks' production of each cow's lactation period.

Hills (*3*) has made a very careful study of the records of 99 cows at the Vermont Experiment Station with regard to the effect of advancing age on the quality of the milk. The records of this herd for a period of 10 years were studied with this question in view both from the standpoint of separately following the same animals through their lives in the herd, making comparisons of groups composed strictly of the same individuals, and also from the standpoint of making comparison of groups according to age, regardless of the changing individuality of the animals. On account of the large number of animals considered, practically the same conclusion was reached in each of the methods of study.

When the same animals were studied throughout their lives in the herd in only 3 out of 46 cases was the percentage of fat higher after the first lactation. Of the remaining 43 cases 29 showed a downward trend of the fat percentage from the first lactation and the remaining 14 showed a stationary fat percentage throughout the period of greatest activity, after which a gradual decline occurred, owing to old age. Hills concludes that—

heifers practically "strike their gait," so far as the quality of the milk flow is concerned, in their first lactation; and whatever the effect of advancing years upon milk quality may be, it is not profound enough to be of importance until old age becomes imminent.

Hooper (5) has recently studied data showing the records of 634 Holstein cows on official test. When the data were classified according to the age of the cows, the percentage of fat in the milk was practically constant from the second through the seventh year. The author concluded that the heifers gave richer milk than the mature cows, but the differences do not appear to be sufficiently great to justify this conclusion. The percentage of fat beginning at 2 years 2 months of age and ending with 7 years 2 months of age was as follows: 3.51, 3.55, 3.54, 3.78, 351, 3.50, and 3.52.

With regard to the question of whether aged cows give normal milk, Klein and Kirsten (6) studied the composition of the milk fat of cows of different ages, the oldest of which was 13 years of age. All the younger cows gave milk fat of normal composition throughout the lactation period, the characteristic changes due to advanced lactation, as manifested in the decline in the saponification value and Reichert-Meissl number and the increase in the iodin value, occurring in each case. The old cow in the group, however, failed to show these changes at the close of the lactation period. The milk fat was also abnormal at the beginning of lactation, showing a Reichert-Meissl number of 43.5 and an iodin value of 31.7. The authors attributed the abnormalities in the case of this cow to individuality rather than to the age of the animal. At the same time they express the view, based on practical experience, that very old cows no longer produce good butter.

INFLUENCE OF AGE ON PERCENTAGE OF FAT IN MILK

The records from which the data presented in this paper are drawn consisted of the true average percentage of fat for the entire lactation period of each cow. This figure has been calculated from the total milk and fat production for the period, the milk production being based on the actual amount of milk produced at each milking for the entire period, and the fat production being based on the percentage of fat in a composite sample of five days' duration taken at the middle of each month. It should also be stated in connection with the lactation periods considered for each animal that whenever this exceeded 12 months the milk and fat production for the 12 months were considered that of the lactation period. This, coupled with the not infrequent failure for periods of parturition to be 12 months apart, results in it being impossible to make the number of lactation periods of an individual animal a criterion of its age. This means, for example, that the 20 Jerseys who completed six lactation periods were not necessarily of the same age at the end of the sixth period. Our data should, therefore, be interpreted more

correctly as representing the influence of the number of lactation periode upon the average percentage of fat for each period. The differencs between the results presented in this form and the actual influence of age upon the percentage of fat did not present the probability of being

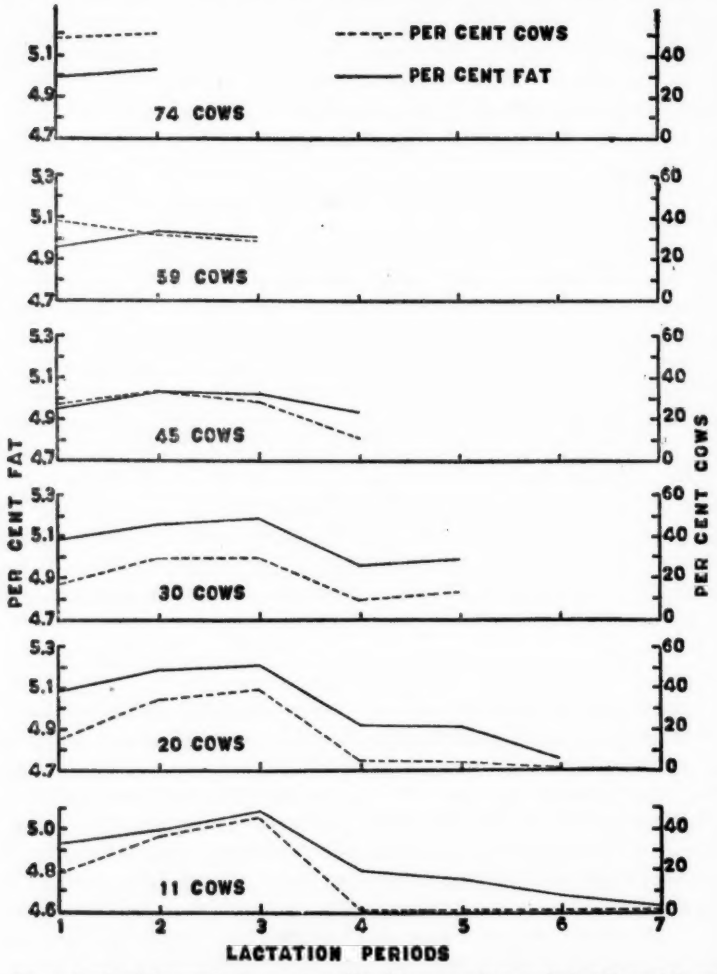

FIG. 1.—Graphs of the variation in percentage of fat of Jersey cows in successive lactation periods, and proportion of total number of cows in each group showing highest test in each period.

of sufficient magnitude to justify the calculation of the data on the basis of the actual age of the animals.

Although the dairy herd from whose records our data are drawn comprised animals of the four breeds already mentioned, not enough animals of the Shorthorn breed were included to warrant the use of

their records from this source. The data, therefore, include the records of animals of the Jersey, Holstein, and Ayrshire breeds only.

The data showing the average percentages of fat for the successive lactation periods of all animals of each breed and similar data for the

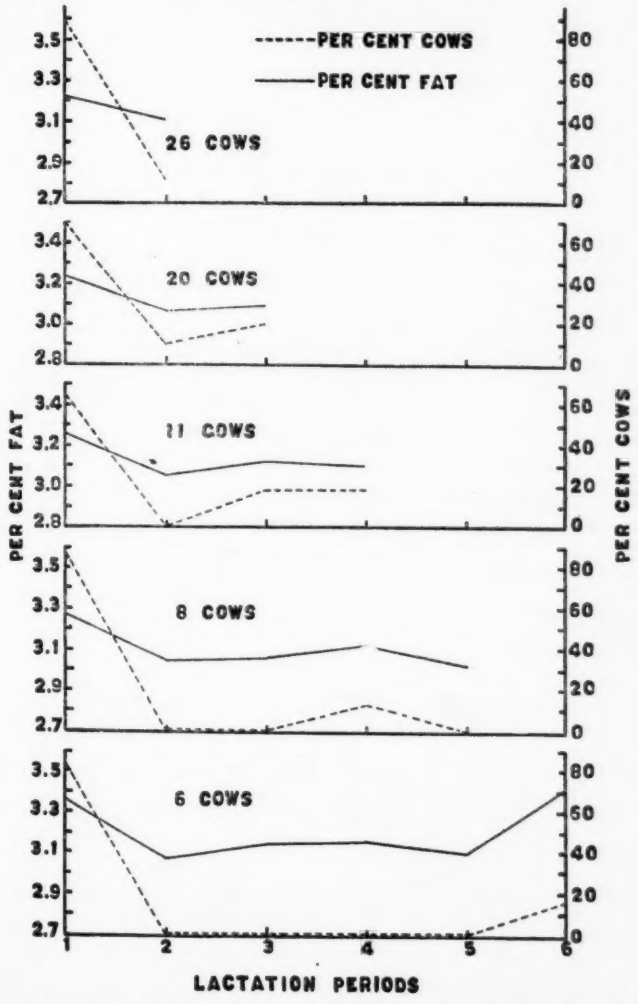

FIG. 2.—Graphs of the variation in percentage of fat of Holstein cows in successive lactation periods, and proportion of total number of cows in each group showing highest test in each period.

entire herd are given in Table I. An examination of the records of the individual animals of each breed, which it would be impracticable to publish on account of their extensiveness, showed rather striking differences among the breeds with respect to the frequency with which the

individuals of each breed showed the highest fat test in each lactation period. This frequency, calculated as the percentage of cows of each group showing the highest test for each period, is presented for each breed in Table II. The combined data for each breed are shown graphically for a portion of the cows in figures 1, 2, and 3.

TABLE I.—*Percentage of fat in milk in successive lactation periods*

JERSEY COWS

Number of cows.	Period—											
	1	2	3	4	5	6	7	8	9	10	11	12
74	4.99	5.04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
59	4.96	5.04	5.01	.....	.....	.....	.....	.....	.....	.....	.....	.....
45	4.95	5.04	5.03	4.84	.....	.....	.....	.....	.....	.....	.....	.....
30	5.09	5.17	5.20	4.97	5.00	.....	.....	.....	.....	.....	.....	.....
20	5.09	5.20	5.22	4.92	4.92	4.76	.....	.....	.....	.....	.....	.....
11	4.93	5.00	5.09	4.80	4.76	4.68	4.63	.....	.....	.....	.....	.....
4	4.89	4.90	5.05	4.68	4.54	4.68	4.61	4.58	.....	.....	.....	.....
3	4.86	4.83	4.83	4.47	4.56	4.64	4.62	4.48	4.47	.....	.....	.....
3	4.86	4.83	4.83	4.47	4.56	4.64	4.62	4.48	4.47	4.22	.....	.....
2	4.99	4.90	4.75	4.50	4.44	4.76	4.70	4.48	4.49	4.33	4.22	.....
2	4.99	4.90	4.75	4.50	4.44	4.76	4.70	4.48	4.49	4.33	4.22	4.01

HOLSTEIN COWS

26	3.22	3.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
20	3.23	3.06	3.09	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	3.26	3.05	3.12	3.10	.....	.....	.....	.....	.....	.....	.....	.....
8	3.27	3.04	3.06	3.12	3.03	.....	.....	.....	.....	.....	.....	.....
6	3.36	3.07	3.15	3.16	3.10	3.41	.....	.....	.....	.....	.....	.....
5	3.33	3.06	3.11	3.11	3.06	3.18	3.11	.....	.....	.....	.....	.....
1	3.29	2.99	3.03	3.08	3.09	3.05	3.00	2.88	.....	.....	.....	.....

AYRSHIRE COWS

9	3.90	3.88	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	3.90	3.88	3.79	.....	.....	.....	.....	.....	.....	.....	.....	.....
6	4.04	3.92	3.81	3.64	.....	.....	.....	.....	.....	.....	.....	.....
4	4.01	3.97	3.78	3.68	3.82	.....	.....	.....	.....	.....	.....	.....
2	4.06	4.08	3.79	3.83	3.84	3.79	.....	.....	.....	.....	.....	.....
1	4.10	3.90	3.87	3.99	3.87	3.76	3.65	.....	.....	.....	.....	.....
1	4.10	3.90	3.87	3.99	3.87	3.76	3.65	3.38	.....	.....	.....	.....
1	4.10	3.90	3.87	3.99	3.87	3.76	3.65	3.38	3.58	.....	.....	.....

ALL COWS

109	4.48	4.48	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
88	4.45	4.47	4.45	.....	.....	.....	.....	.....	.....	.....	.....	.....
62	4.56	4.58	4.58	4.41	.....	.....	.....	.....	.....	.....	.....	.....
42	4.64	4.65	4.66	4.49	4.51	.....	.....	.....	.....	.....	.....	.....
28	4.65	4.66	4.67	4.47	4.45	4.36	.....	.....	.....	.....	.....	.....
17	4.42	4.36	4.44	4.25	4.21	4.18	4.13	.....	.....	.....	.....	.....
6	4.46	4.42	4.52	4.30	4.19	4.26	4.14	4.10	.....	.....	.....	.....
4	4.67	4.60	4.59	4.35	4.44	4.42	4.38	4.21	4.25	.....	.....	.....
3	4.86	4.83	4.83	4.47	4.56	4.64	4.62	4.48	4.47	4.22	.....	.....
2	4.99	4.90	4.75	4.50	4.44	4.76	4.70	4.48	4.49	4.33	4.22	.....
2	4.99	4.90	4.75	4.50	4.44	4.76	4.70	4.48	4.49	4.33	4.22	4.01

In calculating the data in Table II, which may be referred to as a frequency table, it was found in certain cases that a cow showed the same average test in two lactation periods, which usually followed in succession. Those cases in which the average percentage of fat in two periods did not differ more than 0.05 per cent were assigned to this class. In calculating the frequency tables for these cases the assumption was made that the period showing the highest average test meant that no other period showed a higher test, and the frequency calculation was made in favor of the first period which showed the high test.

There are several outstanding features of the tables and figures so far cited, of which mention may be made. In the first place one is impressed by the fact that the differences in the average fat percentage from lactation period to lactation period among the different breeds are not large. Indeed, it is necessary to plot the fat percentages on a rather large scale in order to make apparent the differences which occurred. Inasmuch as the figures do not show all the data given in the tables, it is necessary to turn to them to see that the differences in the average percentage of fat from lactation period to lactation period accumulate to rather large figures as the number of lactation periods of a cow's life becomes greater.

A second striking feature of the data is the marked similarity between the fat percentage and distribution curves. This fact indicates that the distribution curves and the data from which they are drawn are in reality of much greater significance than the curves of percentage of fat. They show clearly that a high percentage of fat in any one period among a large number of cows is due in most cases to the fact that a greater number of cows in that group showed a higher test in that period. This similarity between the curves of fat percentage and distribution of the highest test is especially striking for the 30 Jersey cows shown in figure 1, and for the Holstein cows shown in figure 2.

A third feature of the data is the striking breed characteristic with respect to the frequency of distribution of the highest test in the different lactation periods. In the case of the Jersey cows the data in Table II show that, when only the first two lactation periods are involved, the tendency is for the second period to give a slightly higher average test than the first period, but that this tendency is greater among a few cows than among a large number. In fact, the data show that with the 74 cows having only two lactation periods the distribution was practically equal between the two periods. For cows of the Jersey breed in the herd the tendency for the second period to show the highest average test diminished greatly as the number of lactation periods increased, and in many cases passed to the third period. In general the data show that Jersey cows may be expected to show their highest average test in either the first, second, or third periods, but the chances favor the second and third periods over the first to a rather marked degree. From the third period

on, a gradual diminution of the average test may be expected, and this may accumulate to an appreciable decrease in advanced age. Jersey cows, then, do not necessarily "strike their gait" in the first lactation period with respect to the average percentage of fat in their milk.

TABLE II.—*Relative distribution of highest average test for the lactation period*

JERSEY COWS

Number of cows.	Lactation periods.	Proportion of cows showing highest test in period—						
		1	2	3	4	5	6	7
74	2	48.7	51.3	.....	.....	.....	.....	.....
59	2	47.5	52.3	.....	.....	.....	.....	.....
59	3	39.0	32.2	28.8	.....	.....	.....	.....
45	2	44.5	55.5	.....	.....	.....	.....	.....
45	3	33.3	33.3	33.3	.....	.....	.....	.....
45	4	26.7	33.3	28.9	11.1	.....	.....	.....
30	2	40.0	60.0	.....	.....	.....	.....	.....
30	3	30.0	36.7	33.3	.....	.....	.....	.....
30	4	23.3	36.7	30.0	10.0	.....	.....	.....
30	5	16.7	30.0	30.0	10.0	13.3	.....	.....
20	2	30.0	70.0	.....	.....	.....	.....	.....
20	3	20.0	40.0	40.0	.....	.....	.....	.....
20	4	15.0	40.0	40.0	5.0	.....	.....	.....
20	5	15.0	35.0	40.0	5.0	5.0	.....	.....
20	6	15.0	35.0	40.0	5.0	5.0	0.0	.....
11	2	27.3	72.7	.....	.....	.....	.....	.....
11	3	18.2	36.4	45.5	.....	.....	.....	.....
11	4	18.2	36.4	45.5	0.0	.....	.....	.....
11	5	18.2	36.4	45.5	0.0	0.0	.....	.....
11	6	18.2	36.4	45.5	0.0	0.0	0.0	.....
11	7	18.2	36.4	45.5	0.0	0.0	0.0	0.0

HOLSTEIN COWS

26	2	88.5	11.5	.....	.....	.....	.....	.....
20	2	90.0	10.0	.....	.....	.....	.....	.....
20	3	70.0	10.0	20.0	.....	.....	.....	.....
11	2	100.0	0.0	.....	.....	.....	.....	.....
11	3	81.8	0.0	18.2	.....	.....	.....	.....
11	4	63.7	0.0	18.2	18.2	.....	.....	.....
8	2	100.0	0.0	.....	.....	.....	.....	.....
8	3	100.0	0.0	0.0	.....	.....	.....	.....
8	4	87.5	0.0	0.0	12.5	.....	.....	.....
8	5	87.5	0.0	0.0	12.5	0.0	.....	.....
6	2	100.0	0.0	.....	.....	.....	.....	.....
6	3	100.0	0.0	0.0	.....	.....	.....	.....
6	4	100.0	0.0	0.0	0.0	.....	.....	.....
6	5	100.0	0.0	0.0	0.0	0.0	.....	.....
6	6	83.3	0.0	0.0	0.0	0.0	16.7	.....
5	2	100.0	0.0	.....	.....	.....	.....	.....
5	3	100.0	0.0	0.0	.....	.....	.....	.....
5	4	100.0	0.0	0.0	0.0	.....	.....	.....
5	5	100.0	0.0	0.0	0.0	0.0	.....	.....
5	6	83.3	0.0	0.0	0.0	0.0	16.7	.....
5	7	83.3	0.0	0.0	0.0	0.0	16.7	0.0

TABLE II.—*Relative distribution of highest average test for the lactation period*—Con.

AYRSHIRE COWS

Number of cows.	Lactation periods.	Proportion of cows showing highest test in period—						
		1	2	3	4	5	6	7
9	2	55.5	44.5					
9	2	66.7	33.3					
9	3	55.5	22.2	22.3				
6	2	83.3	16.7					
6	3	83.3	16.7	0.0				
6	4	83.3	16.7	0.0	0.0			
4	2	75.0	25.0					
4	3	75.0	25.0	0.0				
4	4	75.0	25.0	0.0	0.0			
4	5	50.0	25.0	0.0	25.0			

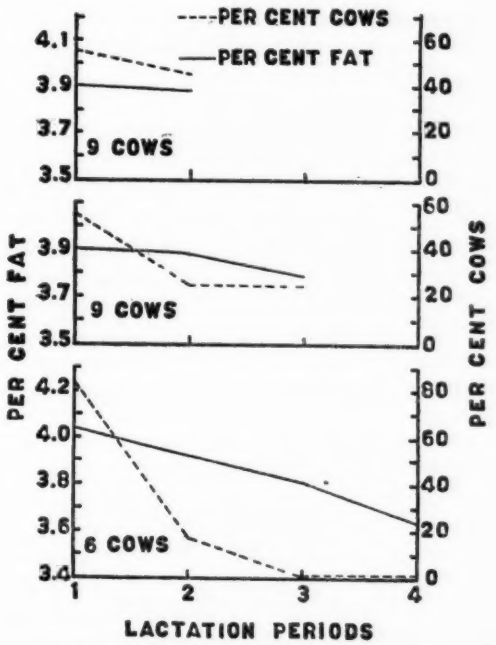

Fig. 3.—Graphs of the variation in percentage of fat of Ayrshire cows in successive lactation periods, and proportion of total number of cows in each group showing highest test in each period.

The data for the cows of the Holstein breed shown in Table II and figure 2 are in marked contrast to those for the Jersey breed. The chances for the highest fat test are so overwhelmingly in favor of the first lactation period that the conclusion seems justified that this is a breed characteristic. A similar tendency is shown by the data from the Ayrshire cows, shown in Table II and figure 3. The tendency is not so

great as for the Holstein cows, but much greater than for the Jersey cows but the data are less conclusive because of the relatively small number of animals involved.

The fact that certain of the animals of the Jersey and Holstein breeds whose records are included in the data comprised an experiment to determine the influences of the plane of nutrition during growth and age of first calving upon the dairy qualities of cows (*1*) suggested that these two factors may have contributed to the difference in the character of the data from the two breeds. If a low plane of nutrition during growth should have a depressing effect upon the percentage of fat in the milk during the first lactation period, as one might expect, this fact would explain to a certain extent why the few animals with the greatest number of lactation periods showed the greatest tendency to have a higher test in the second than in the first period. These animals comprise the oldest animals in the herd; and it is known to be a fact that the heifer calves were not reared on nearly so high a plane of nutrition during the earlier days of the herd than has been practiced in recent years. It may be stated in this connection that the records of the Jersey herd have been kept continuously for a period of 25 years.

The data for the first two lactation periods of the Jersey and Holstein cows used in the above-mentioned experiment have been tabulated in Tables III and IV with respect to the two factors (1) age at first parturition and (2) plane of nutrition during growth.

The data bearing on the age of first parturition, shown in Table III, indicate that a difference of 16 months between the first parturition of the Jerseys had little influence upon the breed tendency already noted for the higher test to accompany the second lactation period. In the case of the Holsteins, however, calving at a more advanced age seemed to decrease the breed tendency for the first lactation period to show the higher average test.

TABLE III.—*Influence of age at first parturition on percentage of fat in milk of first two lactation periods*

Group No.	Number of cows.	Breed of cows.	Average age at first parturition.	Fat in milk.		Cows showing highest test in—		
				First period.	Second period.	First period.	Second period.	Stationary.
			Months.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	22	Jersey	21. 1	5. 04	5. 05	31. 8	45. 5	22. 7
2	21	do	27. 1	5. 10	5. 13	30. 0	70. 0	0. 0
3	16	do	31. 5	5. 09	4. 98	62. 5	31. 2	6. 3
4	14	do	37. 6	5. 17	5. 32	35. 7	50. 0	14. 3
5	4	Holstein	22. 5	3. 29	3. 00	100. 0	0. 0	0. 0
6	12	do	31. 1	3. 21	3. 17	57. 3	16. 7	25. 0
7	9	do	35. 4	3. 19	3. 05	66. 7	11. 1	22. 2

TABLE IV.—*Influence of plane of nutrition during growth on percentage of fat in milk of first two lactation periods*

Group No.	Number of cows.	Breed.	Plane of nutrition during growth.	Fat in milk.		Cows showing highest test in—		
				First Period.	Second Period.	First Period.	Second Period.	Stationary.
				Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1.....	8	Jersey	Heavy fed.....	5. 32	5. 07	62. 5	25. 0	12. 5
2.....	10	.....do.....	Light fed.....	5. 13	5. 21	20. 0	60. 0	20. 0
3.....	7	Holstein.....	Heavy fed.....	3. 35	3. 10	100. 0	0. 0	0. 0
4.....	7	.....do.....	Light fed.....	3. 05	3. 00	42. 8	14. 4	42. 8

The influence of the plane of nutrition during growth, shown in Table IV, is more striking. The data indicate that this factor may be of importance in connection with the average fat test for the first lactation period. The light-fed Jerseys showed both a lower average test in the first lactation period and also a less frequent tendency for this period to show the highest test, while the heavy fed Jerseys showed exactly opposite results. These data seem to justify the tentative explanation already advanced as to why the Jersey group as a whole showed different results from the Holstein and Aryshire groups. The data in Table IV show, in addition, that the strong breed tendency for the Holsteins to show a higher average test in the first lactation period than in any subsequent period was materially diminished by light feeding during the growth of the animals. It may also be pointed out that the light feeding of the animals of both breeds was by no means extreme, although it was widely different from the heavy feeding carried out with the other animals in the experiment.

COMPOSITION AND PROPERTIES OF MILK OF AGED COWS

The fact that certain animals in the Jersey herd of the Missouri Station had the breed characteristic of persistent milking developed to a marked degree has made it possible to examine the composition and properties of the milk of two cows which had attained an advanced age. Complete analyses of the milk fat of cow 124 were made on two occasions in her sixteenth year and during her twelfth lactation period, and again on two occasions in her nineteenth year, when she was in her thirteenth lactation period. Similar analyses were made of the milk of cow 16 when she was 14 years of age, and during her ninth lactation period. Two analyses have also been made of the milk of cow 403, of the Dairy Shorthorn breed, when she was 11 years of age, and during her fourth lactation period. These data are presented in Table V.

TABLE V.—*Influence of extreme age on composition and properties of milk and milk fat*

Cow No.	Age of cow.	Stage of lactation.	Date of sample.	Average daily milk.	Composition of milk.							Composition of fat.			
					Total protein.	Casein.	Albumin and globulin.	Residual nonprotein nitrogen.	Fat.	Lactose.	Ash.	Saponification value.	Reichert-Meissl number.	Iodin value.	Melting point.
	Yrs.	*Days.*		*Lbs.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*			*Hübl.*	*° C.*
[a]124	16	182	June 17–19, 1913	26.3	3.20	2.61	0.59	0.038	4.20	4.65	0.72	232.7	30.82	27.74	33.1
124	16	188	June 24–26, 1913	22.7	3.21	2.62	.59	.039	4.30	5.00	.71	234.2	24.55	29.17	33.1
124	19	838	May 16–18, 1916	7.3	3.11	2.64	.47	.034	4.80	.....	.....	226.0	28.39	37.23	33.3
124	19	[c]864	June 11–13, 1916	12.0	3.64	2.96	.68	.042	4.50	4.32	.51	224.0	26.85	35.64	36.5
[a]16	14	227	June 17–19, 1913	17.1	3.37	2.83	.54	.031	4.15	5.55	.75	231.1	32.74	29.73	35.6
16	14	233	June 24–26, 1913	15.1	3.48	2.95	.53	.039	4.08	5.31	.76	228.9	31.89	31.16	37.0
[b]403	11	155	June 17–19, 1913	23.5	2.88	2.27	.61	.034	3.63	4.91	.70	224.5	24.90	35.12	33.3
403	11	161	June 24–26, 1913	21.5	2.89	2.29	.60	.038	3.63	4.96	.70	225.1	32.38	35.58	33.0

[a] Jersey. [b] Shorthorn. [c] Cow was farrow. Duration of lactation was 1,000 days.

These data fail to reveal any significant features of the composition of either the milk or the milk fat which can be attributed to advanced age. The milk of cow 124 in the nineteenth year of her age failed to show any abnormalities even after she had been in milk for the astonishingly long period of 864 days. It is true that the first analyses of the milk fat made in 1913 in the case of cows 124 and 16 showed a higher Reichert-Meissl than iodin value, as was observed by Klein and Kirsten (*6*) for the milk fat of a cow 13 years of age. Inasmuch as this relation was not borne out in the other analyses no significance can be attached to the two isolated cases.

In order to obtain support for the statement of Klein and Kirsten that old cows no longer give normal butter, a semicommercial churning of butter from cow 124 was carried out with the milk obtained in May, 1916. The cow was on pasture at the time and the butter churned out exceedingly rapidly and was very soft when first made. After hardening, however, it was judged by several persons, including the authors, to be of excellent quality. One-pound portions of the butter were packed in glass jars and placed in the refrigerator. After three months the butter was still of good quality, although it had developed a slightly old taste. It is evident that the statement of Klein and Kirsten does not hold true for all cases, for it was not supported by the case of cow 124, which was much more advanced in years than any of the animals studied by these investigators.

SUMMARY

The percentage of fat in the milk of Jersey cows attains its maximum with respect to the average for the entire lactation period during any one of the first three periods, but the chances appear to be greater that this will be attained in the second or third period rather than the first.

Holstein cows almost invariably show the highest average percentage of fat for the lactation period during the first period.

Ayrshire cows more frequently show a higher average lactation test during the first than during subsequent periods, but less frequently than in the case of Holstein cows.

The variations in the average percentage of fat among the first few lactation periods are not sufficiently great to be of much practical importance, but the gradual decline in average test accumulates to a figure of considerable importance as the number of periods of lactation becomes greater.

A low plane of nutrition during growth and prior to the first lactation period probably contributes materially to a decrease in the average percentage of fat for the first lactation period from that which it would be if the period of growth is supported by a more liberal plane of nutrition.

Neither the percentage composition of the milk nor the physical and chemical constants of the milk fat of aged cows show any abnormalities attributable to old age.

Butter made from the milk of a cow 19 years old and in her thirteenth lactation period was pronounced to be of excellent quality, and kept for a period of three months at a temperature of 8° to 10° C., without showing any marked deterioration.

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SOIL ACIDITY AND THE HYDROLYTIC RATIO IN SOILS[1]

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HISTORICAL REVIEW

Recent investigations pertaining to soil acidity indicate a relationship between iron and aluminium compounds in soils and their reaction to litmus-paper-phenolphthalein indicator. Abbot et al. (*1*)[2] found that soil acidity in certain marsh soils may be due mainly to the presence of aluminum nitrate, and the quantity of aluminum found in an unproductive marsh soil was directly proportional to the amount of alkali required to neutralize the soil solution. Veitch (*9*) and Harris (*6*) found that when acid soils were treated with certain salt solutions iron and aluminium salts came from these soils and the iron and aluminium could be precipitated from the solutions as hydroxids by the addition of alkali. Daikuhara (*4*), working on soils of Japan found a relationship between the acidity of soils and their content of iron and aluminium compounds and concludes that these acid compounds are absorbed by soil colloids. In drawing conclusions from his work Rice (*7*) states:

> much aluminum was present in extracts from soils of the highest acidity,

and Connor (*3*) also concludes that—

> much of the harmful acidity of acid soils is due to the presence of toxic acid salts of aluminum and iron.

Ames and Schollenberger (*2*) state in their conclusions on the subject—

> for the several soils investigated there was found to be an approximately quantitate relationship between the bases soluble in fifth-normal nitric acid (excluding carbonates) and differences in lime requirement by the vacuum method.

A further list of references concerning effects and other causes of soil acidity may be found in the publications already cited and also in those of Frear (*5*) and Truog (*8*), and to avoid repetition the reader is referred to these papers for a more complete discussion of the subject.

THE PROBLEM

From studies on glacial-formed soils the author has for some time observed an apparent relationship between iron and aluminium salts in these soils and their reaction to litmus paper and phenolphthalein indicator. Yellow and brown colored soils of the Glacial Province are often acid to litmus paper. Small, well-marked, highly colored areas of soil

[1] Approved for publication in the Journal of Agricultural Research by the Director of the Michigan Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 672.

in these regions are invariably acid in reaction. Frequently areas of dark-colored acid soils are found having an exceptionally high content of iron or aluminium, or both. Recently an opportunity was given to study the problem from a chemical standpoint, and the results obtained from the investigation are presented in this paper as a contribution to our knowledge of the soil acidity question.

EXPERIMENTAL WORK

EXPERIMENT I.—Twenty-nine samples of miscellaneous soils collected from the several glacial formations of Ingham County, Mich., were analyzed for calcium, iron, aluminium, and silicon by the solubility method, using *N*/5 hydrochloric acid. Averages of duplicate determinations are reported. The reaction of the soils was determined by means of litmus paper in contact with moist soil and also the Veitch qualitative test. The two methods gave similar results on these soils. A summary of the analytical results is given in Table I.

TABLE I.—*Soil classes and quantities of calcium, iron, and aluminium soluble in N/5 hydrochloric acid*

ACID SOILS

[Estimated as oxids. Results expressed as pounds per 2,000,000 pounds of soil]

Laboratory No.	Soil class.	Calcium oxid.	Iron oxid.	Aluminium oxid.
2	Sandy loam	4,270	3,010	5,330
6	Loam	2,390	3,698	3,894
7	Sandy loam	3,100	3,182	2,776
9	Medium sand	1,390	1,462	2,288
10	do	660	1,892	3,132
13	Sandy loam	2,270	3,956	2,144
14	do	2,650	3,612	3,432
16	Medium sand	960	1,720	2,450
17	Clay loam	9,410	4,000	9,356
20	Medium sand	1,300	2,666	3,076
21	do	1,780	2,332	2,750
22	Silt loam	4,170	3,010	3,100
23	Medium sand	1,760	2,708	2,944
24	Sandy loam	4,140	2,880	2,952
27	do	2,040	2,706	1,916
28	do	3,710	2,408	3,294

ALKALINE SOILS

1	Sandy loam	10,780	2,966	5,886
3	do	8,840	2,666	3,220
4	Loam	4,060	3,182	452
5	do	4,750	3,096	530
8	Silt loam	5,860	3,440	3,614
11	do	8,140	3,526	4,298
12	Sandy loam	7,140	3,268	4,062
15	do	6,210	2,966	3,118
18	do	6,380	2,838	3,036
19	do	10,000	3,182	2,988
25	Silt loam	36,000	4,042	9,406
26	do	11,520	2,924	5,041
29	Loam	7,410	2,838	4,310

The results given in the foregoing table do not show striking differences except that the calcium-oxid content of the acid solutions from the alkaline soils is somewhat greater than in the case of the acid soils; but, when ratios between the soluble calcium oxid and iron oxid and aluminium oxid are determined, an interesting relationship is exposed. Table II gives these ratios.

TABLE II.—*Ratios between soluble calcium oxid and iron oxid and aluminium oxid*

ACID SOILS

Laboratory No.	Iron oxid.	Aluminium oxid.	Iron oxid and aluminium oxid.
2	0.70	1.25	1.95
6	1.55	1.63	3.18
7	1.03	.90	1.93
9	1.05	1.65	2.70
10	2.85	4.75	7.60
13	1.74	.94	2.68
14	1.36	1.30	2.66
16	1.77	2.53	4.30
17	.43	.99	1.42
20	2.05	2.37	4.42
21	1.35	1.55	2.90
22	.72	.74	1.46
23	1.54	1.67	3.21
24	.69	.95	1.64
27	1.33	.94	2.27
28	.65	.89	1.54

ALKALINE SOILS

1	0.26	0.55	0.81
3	.30	.38	.68
4	.78	.11	.89
5	.65	.11	.76
8	.59	.62	1.20
11	.43	.53	96
12	.46	.57	1.03
15	.48	.50	.98
18	.43	.46	.89
19	.32	.30	.62
25	.11	.26	.37
26	.25	.44	.69
29	.38	.58	.96

These results show that, although the quantities of calcium, iron, and aluminium soluble in *N/5* hydrochloric acid under the conditions of the method vary considerably in the several soils, there is a marked relationship between the ratios of calcium oxid and iron oxid and aluminium oxid as expressed in Table II and the soil reaction. Considering the ratios $CaO : (Fe_2O_3 + Al_2O_3)$, we find the lowest ratio for an acid soil is 1.42 and the highest ratio for an alkaline soil, 1.20. A neutral ratio may then be approximated by averaging these two numbers, and it is

1.31, or 1.3 nearly. From this standpoint we have a condition in which the ratios $CaO : (Fe_2O_3 + Al_2O_3)$ for the acid soils are all greater than 1 : 1.3, without exception. A similar relationship exists in case of the ratios $CaO : Al_2O_3$, the lowest ratio for an acid being 0.74 and the highest ratio for an alkaline soil 0.62, giving as an average 0.68 for a neutral ratio also without exception. The ratios $CaO : Fe_2O_3$, however, can not be placed in like order without having several exceptions, but if the other ratios can be considered correct because of the absence of exceptions, the correct ratio $CaO : Fe_2O_3$ can be computed and is $1.31 - 0.68$, or 0.63. Three exceptions to this ratio are still apparent—namely, alkaline soils 4 and 5 have ratios greater than 0.63, and the acid soil 17 has a considerable lower ratio. It can be seen, however, that the two alkaline soils mentioned gave exceptionally low quantities of soluble aluminium oxid and the acid soil gave a very high quantity of soluble aluminium oxid. This point is also generally true in case of other soils—that is, alkaline soils having high $CaO : Fe_2O_3$ ratios gave low quantities and acid soils with low $CaO : Fe_2O_3$ ratios gave high quantities of soluble aluminium oxid with few exceptions. Therefore these exceptions can not be considered as invalidating the other results but rather tend to show the close relationship between soluble iron and aluminium in the soils and the soil reaction. There are no exceptions in the ratios between calcium oxid and iron oxid plus aluminium oxid.

The relationship between total calcium, iron, and aluminium was studied by making total analyses in duplicate of the soils for these elements. Ten gm. of soil ground to pass a 100-mesh sieve and 25 gm. of sodium peroxid were fused together in a nickel crucible. After cooling the mass was transferred to a 500-c.c. volumetric flask by means of hot, distilled water, acidified with concentrated hydrochloric acid, and allowed to stand on a steam bath for several hours. The contents of the flask were then cooled to room temperature, brought to a volume of 500 c.c., well mixed, and filtered. The solution was then analyzed according to official methods. The results are given in Table III and the ratios in Table IV.

TABLE III.—*Total quantities of calcium, iron, and aluminium in soils*

[Estimated as oxids. Results expressed as pounds per 2,000,000 of soil]

ACID SOILS

Laboratory No.	Total calcium oxid.	Total iron oxid.	Total aluminium oxid.
2	17,261	82,421	120,984
6	16,154	45,601	139,239
7	14,042	89,531	99,167
9	11,808	39,647	94,184
10	9,553	87,891	99,977
13	19,352	74,087	110,029
14	13,079	74,071	136,349

TABLE III.—*Total quantities of calcium, iron, and aluminum in soils*—Continued

ACID SOILS—continued

Laboratory No.	Total calcium oxid.	Total iron oxik.	Totalaluminium oxid.
16	13,384	82,131	66,008
17	12,874	109,221	217,523
20	13,678	96,851	98,882
21	15,908	79,531	117,478
22	12,505	82,563	131,648
23	14,268	75,656	100,603
24	16,687	100,571	132,287
27	12,546	65,721	110,245
28	15,006	55,866	121,890

ALKALINE SOILS

1	16,236	78,241	155,076
3	19,926	83,391	120,501
4	16,236	41,631	132,972
5	31,652	68,601	139,204
8	15,129	67,455	139,502
11	20,582	73,756	163,434
12	19,106	50,887	129,846
15	27,101	61,221	134,820
18	15,990	75,403	129,359
19	18,163	61,495	127,930
25	44,217	63,483	166,471
26	21,443	53,553	147,943
29	17,056	35,673	113,958

TABLE IV.—*Ratios between total calcium oxid and iron oxid and aluminium oxid in soils*

ACID SOILS

Laboratory No.	Iron oxid.	Aluminium oxid.	Iron oxid + Aluminium oxid.
2	4.78	7.01	11.79
6	2.82	8.62	11.44
7	6.38	7.06	13.44
9	3.36	7.98	11.34
10	9.20	10.46	19.66
13	3.83	5.68	9.51
14	5.66	10.42	16.08
16	6.14	4.93	11.97
17	8.48	16.90	25.38
20	7.08	7.23	14.31
21	5.00	7.38	12.38
22	6.60	10.53	17.13
23	5.30	7.05	12.35
24	6.03	7.93	13.96
27	5.24	8.79	14.03
28	3.72	8.12	8.77

TABLE IV.—*Ratios between total calcium oxid and iron oxid and aluminium oxid soils*—Continued

ALKALINE SOILS

Laboratory No.	Iron oxid.	Aluminium oxid.	Iron oxid + Aluminium oxid.
1	4.82	9.55	14.37
3	4.19	6.05	10.24
4	2.56	8.19	10.75
5	2.17	4.40	6.57
8	4.46	9.22	13.68
11	3.58	7.94	11.52
12	2.66	6.80	9.46
15	2.26	4.97	7.23
18	4.72	8.09	12.81
19	3.39	7.04	10.43
25	1.44	3.76	5.20
26	2.50	6.90	9.40
29	2.09	6.68	8.77

While these results show that, in general, the total quantities of calcium and aluminium are lower and the total quantities of iron higher in the acid than in the alkaline soils, there is no distinct relationship between these total quantities and the soil reaction as there is in the case of the acid-soluble portions.

Total sulphur and magnesium were determined also, but no relationships were found between these elements and the soil reaction. The largest quantity of magnesium found in an acid soil was 3,226 pounds per 2,000,000 pounds of soil, and for an alkaline soil 4,288 pounds: consequently these soils may be characterized as low in magnesium.

As stated before, the acid-soluble silica was determined and these results, together with the "lime requirement" of the acid soils determined by the Veitch method, are given in Table V. A certain but not well-defined relationship exists between the quantities of acid-soluble silica and the "lime requirement." No other relationships concerning acid-soluble silica were discovered.

TABLE V.—*Quantity of acid-soluble silica (SiO_2) in 2,000,000 pounds of the soils and also the "lime requirement" of the acid soils by Veitch method*

Acid soils.			Alkaline soils.	
Laboratory No.	SiO_2.	Lime requirement.	Laboratory No.	SiO_2.
	Pounds.	*Pounds.*		*Pounds.*
2	1,020	1,560	1	1,570
6	960	1,320	3	1,330
7	670	1,440	4	1,230
9	620	1,440	5	1,430
10	520	1,440	8	1,380
13	730	1,440	11	2,280
14	960	1,560	12	1,490
16	540	1,440	15	1,010
17	2,520	3,600	18	1,370
20	780	1,200	19	1,460
21	640	1,080	25	3,480
22	1,110	1,560	26	2,060
23	640	1,440	29	850
24	1,020	1,800		
27	590	1,320		
28	730	1,560		

From the data given in Tables I and II it is evident that the relationship between the acid-soluble calcium and iron and aluminium may control the soil reaction. The question is, then, under what conditions can this phenomenon be produced. It is well known that many calcium salts hydrolyze in solution giving an excess of base, and reacting alkaline to litmus indicator, and that iron and aluminium salts generally produce acid solutions under similar conditions. The reaction of a solution containing a mixture of a salt that hydrolyzes and gives an excess of hydrion with a salt that produces an excess of hydroxidion on hydrolyzing depends upon the concentration and degree of hydrolysis of each salt in the presence of the other when equilibrium is established. The term "hydrolytic ratio" is used here to express this relationship. In other words, if the acid exceeds the alkali by chemical equivalents, the solution will react acid, and an alkaline solution results on reversing the conditions, while a certain hydrolytic ratio gives a neutral solution.

Results obtained from the analytical determinations with the solubility method and the theory of hydrolysis give a basis for the following hypothesis: that the reaction of these soils is probably determined chiefly by the hydrolytic ratio between salts of the alkaline earth elements on the one hand and salts of iron and aluminium on the other when these compounds are in equilibrium. From this standpoint any hydrolyzable salt in a soil or added to a soil, or any salt added to a soil that causes the formation of a hydrolyzable salt, may influence the soil reaction. In the following pages other data are given to support the above hypothesis.

EXPERIMENT II.—The quantity of lime required to neutralize these soils was determined by indirect titration with limewater in the following

manner: 6–10, 300-c. c. Erlenmeyer flasks were used for each soil, 1 gm. of air-dried soil was placed into the first flask and the quantity increased in the remaining flasks by increments of 1 gm. each. Fifty c. c. of distilled water were then added to each flask and ten c. c. of a standardized lime-water solution. The flasks were sealed immediately with rubber stoppers, shaken several times, and allowed to stand about 16 hours. End points were determined in two ways: (1) by means of electrical resistance, and (2) with phenolphthalein indicator. Resistance measurements were taken by removing 25 c. c. of the supernatent solution with a pipette to a resistance cup and the readings taken with a Kohlrausch bridge. The highest resistance reading in the series was taken as the end point. A drop of indicator was then placed into each flask and the end point taken when the red color showed faintly. In all cases with the soils under discussion there was exact agreement in the end points as determined by the two methods.

On considering the ratio $CaO : Fe_2O_3 + Al_2O_3 :: 1 : 1.3$ to be correct for a neutral soil, the "lime requirement" of the acid soils may be computed by determining the quantities of calcium oxid necessary to add to the calcium oxid found soluble in the soils to bring all the ratios to 1:1.3. Table VI contains these figures, together with the "lime requirement" as determined by indirect titration. The quantities of acid-soluble calcium oxid plus the quantities of calcium oxid required to neutralize the soils by indirect titration are given in the fourth column, and the last column contains the ratios between these quantities of calcium oxid and the quantities of acid-soluble iron and aluminium.

TABLE VI.—*Lime requirement and ratios produced by neutralizing acid soils*

[Expressed as pounds per 2,000,000 pounds of soil.]

Laboratory No.	Calcium oxid required from neutral ratio.	Calcium oxid required by titration.	Acid-soluble calcium oxid + calcium oxid required by titration (=X).	$\frac{Fe_2O_3 + Al_2O_3}{X}$
	Pounds.	*Pounds.*	*Pounds.*	
2	2,140	2,040	6,310	1.32
6	3,450	3,240	5,630	1.35
7	1,480	1,600	4,700	1.27
9	1,490	1,600	2,990	1.25
10	3,210	3,140	3,800	1.32
13	2,420	2,400	4,670	1.31
14	2,770	2,800	5,450	1.29
16	2,250	2,200	3,160	1.30
17	860	940	10,350	1.29
20	3,120	2,800	4,100	1.40
21	2,120	1,900	3,680	1.38
22	530	660	4,830	1.27
23	2,590	2,160	3,920	1.44
24	1,220	1,160	5,300	1.29
27	1,520	1,400	3,440	1.34
28	580	660	4,370	1.30

In Table VII is shown the excess of acid-soluble iron oxid and aluminium oxid in the acid soils over the amount required to satisfy the ratio $CaO : Fe_2O_3 + Al_2O_3 :: 1 : 1.3$, and the ratios between the calcium oxid required by indirect titration and this excess of iron oxid plus aluminium oxid.

TABLE VII.—*Excess (in pounds) of iron oxid plus aluminium oxid over ratio 1 to 1.3 and ratios of calcium oxid required by titration to excess of iron oxid plus aluminium oxid*

Laboratory No.	Excess of iron oxid plus aluminium oxid.		Laboratory No.	Excess of iron oxid plus aluminium oxid.	
	Over 1:1.3.	Ratio of calcium oxid by titration.		Over 1:1.3.	Ratio of calcium oxid by titration.
	Pounds.			*Pounds.*	
2	2,790	1.37	17	1,122	1.19
6	4,485	1.38	20	4,052	1.45
7	1,928	1.21	21	2,758	1.45
9	1,943	1.22	22	690	1.05
10	4,166	1.33	23	3,364	1.56
13	3,149	1.31	24	1,449	1.25
14	3,599	1.29	27	1,970	1.41
16	2,922	1.33	28	879	1.33

It may be seen from Table VI that the "lime requirement" as determined from the neutral-soil ratio and by indirect titration closely agree, causing the neutral ratio for all the acid soils to approximate 1:1.3 also, as shown by Table VII, when the lime required to neutralize the acid soils is compared to the excess of iron oxid plus aluminium over the ratio 1:1.3, these ratios closely approach the same figure. The relationship between acid-soluble calcium and iron and aluminium compounds in these soils and the soil reaction is clearly shown, and it is also evident that we are dealing with equilibrium conditions.

EXPERIMENT III.—This experiment was performed to determine, if possible, the nature of the equilibrium—that is, whether physical or chemical. Soil 23 was chosen, the procedure being similar to that used in determining "lime requirements" by the indirect-titration method, except that after standing, the several portions were filtered, the soil washed with cold distilled water, and the filtrates titrated with an acid solution equivalent to the limewater solution used, with phenolphthalein as indicator. The experiment was performed in two ways: (1) By keeping the quantities of soil constant and varying the quantities of calcium oxid in contact with the soil, and (2) by varying the quantities of soil but keeping those of calcium oxid constant. The concentrations change, however, when the soil begins to take up lime. The results are given in Table VIII.

TABLE VIII.—*Effects of an excess of a solution of calcium hydroxid on the amount of calcium oxid used by acid soil 23*

VARYING QUANTITIES OF CALCIUM OXID, SOIL CONSTANT

Erlenmeyer flask No.	Weight of soil taken.	Quantity of calcium hydroxid taken.	Quantity of water added.	Titer of filtrate (cubic centimeters of hydrochloric acid).	Quantity of calcium hydroxid used by soil.	Percentage of applied calcium hydroxid.	Quantity of calcium oxid used per 2,000,000 pounds of soil.
	Gm.	*C. c.*	*C. c.*		*C. c.*		*Pounds.*
1	5	2	10	.....	2.0	100	.....
2	5	4	20	.....	4.0	100	.....
3	5	6	30	0.2	5.8	97	2,320
4	5	8	40	.6	7.4	92	2,960
5	5	10	50	2.4	7.6	76	3,040
6	5	12	60	3.6	8.4	70	3,360
7	5	14	70	5.5	8.5	61	3,400
8	5	16	80	7.7	8.3	52	3,320

VARYING QUANTITIES OF SOIL, CALCIUM OXID CONSTANT

1	1	3	20	0.75	2.25	75.0	4,500
2	2	3	20	.25	2.75	91.6	2,748
3	3	3	20	.1	2.9	96.6	1,930
4	4	3	20	.....	3.0	100.0	.....
5	5	3	20	.....	3.0	100.0	.....

It is evident that the soil continues to take up calcium oxid after the solution becomes alkaline to phenolphthalein, but the quantity used (Table VIII) soon reaches a maximum and remains quite constant. Soil acidity is undoubtedly neutralized when an excess of calcium oxid is found in the solution. A second reaction must then take place which is complete when the maximum amount of calcium oxid is taken. It will be observed from the table that this maximum amount increases with the concentrations of the solutions in contact with the soil. The quantity of calcium oxid required to neutralize 2,000,000 pounds of soil 23, as determined by the indirect-titration method with a different concentration of limewater than was used in Experiment III, is 2,160 pounds, an amount comparable to that used by the soil in this experiment when the solution becomes slightly alkaline.

Two generalizations can be drawn from the data given:

(1) A definite quantity of calcium oxid is required to neutralize an acid soil, with phenolphthalein as the indicator; and this quantity is independent of the concentration of the limewater used.

(2) Beyond the neutral point the amount of calcium oxid used varies directly with the concentration of the limewater solution bathing the soil; hence, it appears that the first generalization deals with a chemical reaction, and the second with a physical reaction. However, mass action may cause a chemical reaction when an excess of calcium oxid is present.

At this point the possible combinations of the alkali earth elements and iron and aluminium with other elements in soils may well be considered. The principal mineral salts of the alkali earths are carbonates, silicates, phosphates, sulphates, chlorids, and nitrates. Of this list only the carbonates and silicates of calcium and magnesium give a red color on boiling their solutions with phenolphthalein. The principal iron and aluminium salts found in soils are silicates and phosphates, while sulphates, chlorids, and nitrates may be present. Theoretically these salts hydrolyze and give acid solutions, and will react with carbonates of silicates of the alkali earths; but these reactions proceed to an equilibrium and not to the limits of chemical equivalents.

EXPERIMENT IV.—Dilute solutions of the carbonate and silicate of calcium were boiled with phenolphthalein until the red color appeared. Small quantities of iron and aluminium salts were then added until the red color disappeared. Several salts of iron and aluminium were used in this manner, and the red color could be obtained or dispelled at will by increasing in order the calcium or iron and aluminium salts. Similar results were obtained by using solutions from alkaline and acid soils.

If a soil solution gives a red color with phenolphthalein when boiled and concentrated, a free alkali hydroxid is undoubtedly present, probably coming from the decomposition of a carbonate or silicate of an alkali earth element. On the other hand, if a soil solution gives no red color under the above-named conditions, an acid salt may be present in sufficient quantity to prevent the formation of free alkali.

From the ratios given in the preceding pages two general conclusions can be drawn: (1) The quantities of acid-soluble calcium are proportional to the quantities of acid-soluble iron and aluminium in neutral soils, and also to the unreacting acid-soluble iron and aluminium in the acid soils; and (2) the quantities of calcium required to neutralize the acid soils are proportional to the quantities of acid-soluble iron and aluminium in excess of the ratio 1:1.3 or to the reacting iron and aluminium. These results can not be explained on the basis of absorption, unless iron and aluminium compounds are the only absorbing substances in the soils, or that these compounds are proportional in acid-soluble quantities to the total amounts of absorbing compounds; also, if soils contain other absorbing substances, their absorbing power is satisfied to an equal degree; or that $N/5$ hydrochloric acid dissolves an amount of absorbed lime proportional to the unreacting iron and aluminium in the acid soils; and also soils exhibit two kinds of absorption, one kind independent of the concentration of limewater and the other dependent upon it. According to present knowledge of soil composition and absorption, these considerations are untenable.

The proportions found are in direct accord with the theory of hydrolysis and chemical equilibrium. When a neutral equilibrium is established between hydrolyzing compounds, the reacting substances bear certain

fixed relationships to each other, which may be expressed in the form of a proportion. An equilibrium not neutral in reaction requires the addition of a quantity of acid or aklali proportional to the uncombined substances before the neutral point is obtained. If a solution of an acid salt of iron or aluminium is titrated with a standard solution of limewater until the reaction proceeds to a certain point but not to neutrality, the quantity of calcium oxid used is proportional to the quantity of iron or aluminium in the portion of the salt decomposed, and the amount of calcium oxid yet required to neutralize the solution is in the same proportion to the quantity of iron or aluminium in the portion of the salt yet to be acted upon, as is also the total quantity of iron or aluminium in the salt neutralized, a condition similar to that found in the acid-soluble portions of the soils studied.

When determinations for "acid consumed" were made on the soils preliminary to the solubility method, it was observed in case of the medium sands that more alkali was required to neutralize the acid solutions than was necessary to combine with the quantity of $N/5$ hydrochloric acid used; therefore these soils may have contained acid salts soluble in the acid in order to require this excess of alkali to neutralize the solutions.

The reddening of blue litmus paper by soils has been explained from the standpoint of selective absorption, but the phenomenon can be as logically explained on the basis that these soils contain acid salts absorbed by soil gels or relatively insoluble. Phosphates and hydrated silicates of iron or aluminium can be washed with water until the filtrates do not react acid to litmus paper, but when the paper is brought in contact with the solid material it is strongly reddened. It is also well known that soils which do not redden blue litmus paper, as well as neutralized acid soils, absorb large quantities of calcium oxid from limewater solutions. Silica gels also hold soluble-acid salts of iron or aluminium so tenaciously that these salts can not be washed out. The fact that a soil solution is not acid to litmus is no proof that an acid salt does not exist in the soil.

From the results obtained it is believed that the indirect-titration method as previously explained is adequate to determine the quantity of lime required to neutralize an acid soil. In determining the lime requirement of an acid soil due regard should be given to the factors influencing salt hydrolysis, solubilities of soil minerals, and mass action. Time, temperature, and concentrations of the reacting compounds are the principal factors involved. It is well known that in reactions concerning mass action, hydrolysis, and solubilities a certain length of time is required for equilibrium. Because of the effects of temperature on hydrolysis and solubility, a temperature considerably above or below that normal to field soils under plant-growing conditions should probably not be used. A point of importance in the procedure of the indirect-titration method is that the quantities of reacting substances, soil and

limewater, are not changed during the reaction. Generally speaking, quantities of the reacting compounds are chosen that give a neutral equilibrium at a normal temperature. In this way the true point of neutrality is obtained, a fact undoubtedly known but not fully appreciated by many investigators, because in investigations on the subject and in developing lime-requirement methods an excess of soil or reagents is commonly used without apparent regard to the effects of mass action. Consideration should also be given to the nature of the products formed during the reaction. It will not be sufficient to determine the degree of acidity in a soil and calculate the amount of lime required to neutralize this acidity, because soil acidity undoubtedly results from the hydrolysis of soil compounds and enough reagent should be used to force the hydrolytic action to the neutral point. If calcium carbonate is used as a reagent, it should be in a very finely divided condition; otherwise the particles become coated with gels of iron, aluminium, or silica at the point of reaction with the soil, and their rate of solution is retarded. Considering all the aspects of the case, the logical reagent to use in a lime requirement method is calcium hydroxid.

SUMMARY

(1) A definite relationship was found between the ratios of calcium to iron and aluminium soluble in $N/5$ hydrochloric acid and the soil reaction. All the acid soils have ratios $CaO : (Fe_2O_3 + Al_2O_3)$ above 1 : 1.3, and all the alkaline soils have ratios below this figure.

(2) It is believed that the reactions of the soils studied depend chiefly upon the hydrolytic ratios existing between hydrolyzing compounds of the alkali earths and iron and aluminium.

(3) A method for determining the calcium oxid required to neutralize a soil by indirect tiration is described in which certain fixed quantities of a standardized calcium-hydroxid solution are allowed to react separately with varying amounts of soil. The concentrations of soil and solution giving a neutral reaction are chosen from the series by comparing the electrical resistance of the several solutions, also by means of phenolphthalein indicator. The greatest electrical resistance and faint color of the indicator is coincident with the concentration giving a neutral reaction.

(4) The quantities of lime required to neutralize the acid soils may be determined by computing the quantities of calcium oxid necessary to add to the acid soluble calcium oxid found in the soils to bring the ratios $CaO : Fe_2O_3 + Al_2O_3$ to 1 : 1.3. The quantity of calcium oxid required by this factor method corresponds closely to the quantities required when determined by means of the indirect titration method, and it appears that the titration method is accurate and convenient.

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